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MERTON'S METHOD OF MEASURING THE HALF-WIDTH OF A SPECTRAL SOURCE AND THE FABRY AND PEROT ETALON[1]

By J. K. Robertson[2]

Abstract

A discussion is given concerning the possibility of applying to a Fabry and Perot étalon, Merton's method of evaluating the half-width of a spectral source. Using sources of known half-width as determined from Doppler effect and kinetic theory considerations, the writer has plotted the exact distribution of intensity in the image obtained with an étalon of about one centimetre thickness. By means of the resulting graphs a comparison is made between results obtained with the use of rigid theory and with a simplification similar to that previously used by Merton for another instrument. It is shown that the simplified method cannot in general be applied to an étalon.

Introduction

Since, in the case of a luminous gas at low pressure, the half-width of a spectral source is a function of the mass of an individual radiator, measurements of this quantity have been of great importance in many investigations. Experimentally the value of the half-width has been obtained in several ways.

(a) By deduction from visibility curves in accordance with the pioneer work of Michelson (9).

(b) By observation of the maximum path difference for which interference fringes can be obtained. This method has been used to a considerable extent by Fabry and Buisson (1, 2, 5).

(c) By a method due to Burger and Van Cittert (3) in which the true half-width of a source is deduced from measurements of the apparent half-width of photographic images made with at least two different settings of the interferometer.

(d) By obtaining, with a high power interference spectroscope, two photographs under conditions which permit of a known intensity change.

The last method, due to Merton (7, 8) and as far as the writer is aware, used only by him, differs from all the others in the fact that by means of it the value

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of the half-width of a source is obtained from observations made with only one setting of the interferometer. It has the disadvantage however that, in using it, it is an extremely difficult matter to apply rigid theory. In the paper in which the method is described by Merton, use was made of simplified theory apparently applicable to an echelon diffraction grating. The rigid treatment for this instrument was worked out by the writer (11) who showed that there was some justification for the simplification used by Merton.

As the Fabry and Perot étalon is another instrument suitable for the use of Merton's method, it seemed desirable to examine for this instrument the possibility of replacing rigid theory by an approximation similar to that used by Merton in the case of the grating. In this paper it is shown that in general this cannot be done.

Fundamental Theory

For the sake of clearness, a brief reference must be made to fundamental ideas in connection with half-width problems. In the case of a radiating gas at low pressure, if we assume the Doppler effect as the chief cause for departure from monochromatism, the energy corresponding to a single spectral line is distributed among a small range of wave-lengths according to the law

$$E = E_0 e^{-k^2 x^2} , \qquad (1)$$

where E_o is the intensity of the central wave-length λ_o, where x, a function of the wave-length λ, is defined by the relation

$$\frac{1}{\lambda} = \frac{1}{\lambda_0}\left(1 \pm x\right), \qquad (2)$$

and where k^2 is a function of m, the mass of a single radiator, and of T, the absolute temperature, in accordance with relations (3) and (4) below.

The half-width $\delta\lambda$ of the source is defined as that given by the value of x for which the intensity has fallen 50%, that is, for which $E = 0.5\ E_o$. It follows from equations (1) and (2) that

$$\left(\frac{\delta\lambda}{\lambda}\right)^2 = \frac{\log_e 2}{k^2} . \qquad (3)$$

Since, according to the pioneer work of Rayleigh (10) and Schönrock (13)

$$\frac{\delta\lambda}{\lambda} = 3.57 \times 10^{-7} \sqrt{\frac{T}{m}}, \qquad (4)$$

a knowledge of $\delta\lambda$ (or of k^2) leads to an evaluation of m.

Merton's Method

Merton's method for the evaluation of k^2 is based on the following considerations. The distribution of intensity in the spectral image formed by an instrument such as a Fabry and Perot étalon is given by

$$I = I_o f(\phi, k^2) , \qquad (5)$$

where ϕ is the angular distance from the region of maximum intensity I_o at the centre of the image. It is important to note that equation (5) has reference to the distribution of intensity in an actual image. This image is the result of two causes: (a) the conditions imposed on the light by the instrument: (b) the lack of monochromatism of the source. Even with absolutely monochromatic

light ($k^2=\alpha$), there is a distribution of intensity over a finite width. Equation (5), therefore, must not be confused with equation (1).

In photographing such an image (that is, the interference system of the étalon), there is always a critical intensity which, in a given time, just produces a visible blackening on the plate. This of course corresponds to the edges of the image (or the edges of a bright fringe). If I_c be such a critical intensity and ϕ_c the corresponding value of ϕ,

$$I_c=I_0 f(\phi_c, k^2). \qquad (6)$$

Suppose, now, that two photographs are taken under such conditions that I_o is changed by a known ratio from I_1 to I_2. Then

$$\begin{aligned} I_c &= I_1 f(\phi_{c1}, k^2) \\ &= I_2 f(\phi_{c2}, k^2), \end{aligned}$$

or

$$I_1 f(\phi_{c1}, k^2)=I_2 f(\phi_{c2}, k^2). \qquad (7)$$

Relation (7) provides the basis of Merton's method, for, using the observed values of ϕ_{c1} and ϕ_{c2}, and the known ratio of I_1 to I_2, one can theoretically evaluate k^2, and hence find $\delta\lambda$.

In applying the method, the difficulty lies in the fact that the form of the function in relation (6) or (7) is far from simple. In the case of the étalon, Saha (12) and Dempster (4) and, more recently, Gilchrist (6) have shown that the law of distribution of intensity in the image is given by

$$I=constant^* \left\{ 1+2\,r^2.\cos\delta.\,e^{-\frac{p^2\pi^2}{k^2}} +2\,r^4.\cos 2\delta.\,e^{-\frac{2^2p^2\pi^2}{k^2}} + - - - - - - - \right\}, \qquad (8)$$

where r^2 = coefficient of reflection of the half-silvered surfaces, p = the order of interference, k^2 has the same meaning as in equation (1), and $\delta=2\pi p$.

Since at the centre of the image (or bright fringe) p is an integral number, the variation in intensity as one goes from the centre of one bright fringe to the next can be found by giving δ values from 0° to 360°, and plotting relation (8). Examples of the resulting graphs for particular values of r, p, and k will be found in Fig. 1, curves 1 and 2, and Fig. 2, curves 1 and 2.

From an actual photograph, values of the angle ϕ are obtained, but it is not a difficult matter to apply the simple theory of an étalon and to calculate the corresponding values of δ. In the work which follows, it should be remembered that ϕ and δ are in this sense interchangeable.

In the paper in which the writer discusses the application of Merton's method to the echelon, it is shown that this is done by substituting the exponential factor

$$e^{-k^1\phi^2} \qquad (9)$$

for a complicated expression somewhat analogous to that in relation (8). In so doing he is therefore assuming that graphs such as 1 and 2, Fig. 1, which accurately represent equation (8), are exponential, or that an exponential distribution in the source gives rise to an exponential distribution in the actual

**This constant is a function of r^2 but not of δ and hence its value need not be known in order to plot the intensity distribution curve.*

image. On the face of it, this cannot be true generally because, as already pointed out, there is a finite width of the image even for absolutely monochromatic light. Within certain limitations, however, the error was not great in the case of the echelon. Is this also true for the étalon ?

Tests

In considering the tests which were made to answer that question, it is necessary to distinguish carefully between the k of equation (1) and the k^1 in Merton's exponential factor. As is evident from equations (1) and (3), k is a measure of the half-width of the *source*. k^1 is defined in terms of the half-width of the actual *image* and has a magnitude given by

$$k^1 = \frac{\log_e 2}{(\delta\alpha)^2} \tag{10}$$

where $\delta\alpha$ is the value of ϕ for which $e^{-k^1\phi^2}$ has fallen 50%.

Test No. 1

(a) Assuming the correctness of Rayleigh's theory as given by equation (4) we have, for the hydrogen molecule, at $T=456°$A,

$$\frac{\delta\lambda}{\lambda} = 0.53\times10^{-5} \quad ,$$

from which, by equation (3),

$$k^2 = 2.25\times10^{10} \; .$$

For an étalon with distance between the half-silvered surfaces equal to 1 cm., p is of the order of 40,000. Taking a possible value of r^2*, such as 0.8, we can then plot the correct distribution of intensity in the image by giving δ successive values in equation (8). The resulting curve is given in graph 1, Fig. 1.

By means of this graph it can be seen that the intensity has fallen to 50% of the maximum for a value of $\delta=90°$. This, then, is the value of the half-width of the image obtained by the use of the rigid theoretical expression.

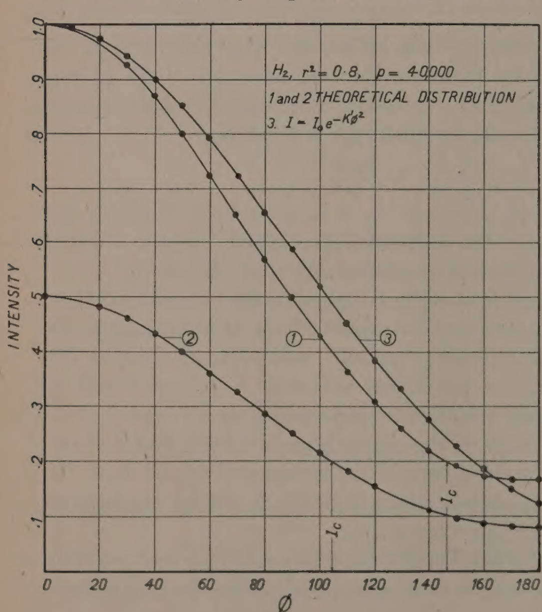

FIG. 1. *Graphs showing the distribution of intensity in a bright fringe for an étalon, when the radiator is a hydrogen molecule; (1) and (2) by the use of rigid theory; (3) by Merton's approximation.*

Suppose, now, we apply Merton's method and use the simple exponential factor in (9). Then, if ϕ_1 and ϕ_2 are the values of ϕ corresponding to the critical intensity, for maximum intensities I_1 and I_2 respectively,

**Half-silvered surfaces for which this is the value of r^2 are very satisfactory.*

$$I_c = I_1 e^{-k^1\phi_1^2} = I_2 e^{-k^1\phi_2^2} \quad ,$$

from which

$$k^1 = \frac{\log_e \dfrac{I_1}{I_2}}{\phi_1^2 - \phi_2^2} \quad . \tag{11}$$

In Fig. 1, graph 2 has been obtained by halving all the intensities of graph 1, thus making $I_1 = 2I_2$. Assuming, then, any reasonable value for I_c*, such as 0.2 on the arbitrary scale of the graphs, we can read off $\phi_1 = 146°$, $\phi_2 = 104°$. It is then found from equation (11) that

$$k^1 = 0.0000659.$$

Finally, from equation (10) we obtain for the value of the half-width of the image by this method

$$\delta\alpha = 102.5°.$$

(b) A similar test was made using the element Hg and the values $k^2 = 2.25 \times 10^{12}$, $r^2 = 0.8$, and $p = 37{,}120$. This value of p was chosen because it is the order of interference for mercury wave-length 5461 in the case of an étalon on which some measurements of the fringe system had been previously made.

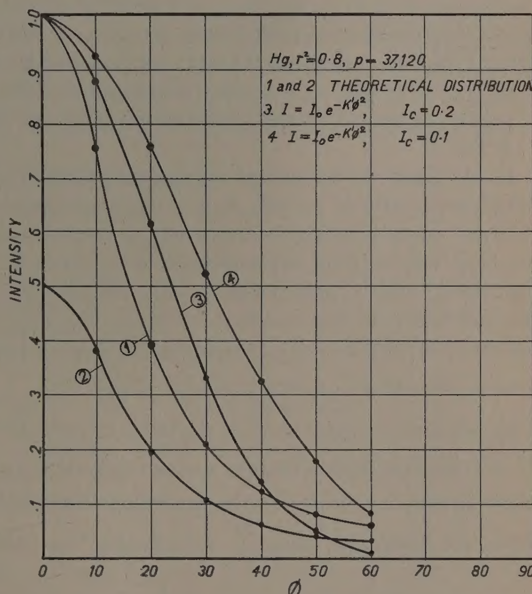

Fig. 2. *Graphs showing the distribution of intensity in a bright fringe for an étalon, when the radiator is a mercury atom; (1) and (2) by the use of rigid theory; (3) and (4) by Merton's approximation.*

Graphs 1 and 2 of Fig. 2 again give the exact theoretical distribution of intensity, with $I_1 = 2I_2$. In calculating numerical values for these curves it may be pointed out that, for each value of δ, 15 terms of the intensity distribution expression (8) were used.

From either of these graphs, it is seen that

$$\delta\alpha = 16.5°.$$

If, again, we take $I_c = 0.10$, we find $\phi_1 = 44.1°$, $\phi_2 = 30.7°$, and hence, by equation (11),

$$k^1 = 0.000691$$

and, by equation (10)

$$\delta\alpha = 31.7°.$$

**The value of ϕ_c depends on the time of exposure. In an actual plate examined by the writer, ϕ_c, for a fringe system of the mercury wave-length 5461 taken with an étalon, had a value of 57° (expressed in terms of δ). From graph 1, Fig. 2, it will be seen that this corresponds to $I_c = 0.09$.*

Repeating this work with $I_c = 0.20$, we obtain $\phi_1 = 30.7°$, $\phi_2 = 19.4°$, $k^1 = 0.001223$ and

$$\delta\alpha = 23.8°.$$

A repetition of the above with $r^2 = 0.75$, $I_c = 0.10$, $p = 37{,}120$, gave $\phi_1 = 44.1°$, $\phi_2 = 40.2°$, and

$$\delta\alpha = 19.5°.$$

A slight variation of this method of finding $\delta\alpha$ consists in using the values of k^1 obtained above to plot the exponential curves

$$I = I_0 e^{-k^1\phi^2} .$$

From the resulting graphs (curve 3 of Fig. 1 corresponding to $k^1 = 0.0000659$; curve 3 of Fig. 2 to $k^1 = 0.000691$; curve 4 of Fig. 2 to $k^1 = 0.001223$) values of ϕ (or δ) for which the intensity has dropped 50% give values of $\delta\alpha$. These, of course, agree with those given above (that is, 102.5°, 31.7° and 23.8°).

Test No. 2

In applying his simplified method to the echelon, Merton's procedure consisted essentially in making actual measurements of the ϕ_1 and ϕ_2 of equation (11) and in calculating $\delta\alpha$ by means of equations (10) and (11). The corresponding half-width of the source, that is $\delta\lambda$, was then found on the assumption that the $\Delta\lambda$ which corresponds to $\delta\alpha$ in ordinary echelon theory correctly defines the half-width of the source as given by equation (1). If we make the same assumption with regard to the étalon, it follows that

$$\frac{\delta\lambda}{\lambda} = \frac{\delta\alpha}{2\pi} \cdot \frac{1}{p}. \qquad (12)$$

This relation provides us with the basis of another test, for, again taking values of $\frac{\delta\lambda}{\lambda}$ from Rayleigh's theory, we can calculate $\delta\alpha$ from equation (12) and see what agreement there is with the values obtained by the use of rigid theory. Thus, for hydrogen, using $\frac{\delta\lambda}{\lambda} = 0.53 \times 10^{-5}$ as before, and $p = 40{,}000$ we have

$$\delta\alpha = 76.3° ,$$

compared with 90° from rigid theory, and with 102° by the other method based on Merton's theory as described in Test No. I.

A similar test in the case of Hg, with values of $\frac{\delta\lambda}{\lambda} = 0.053 \times 10^{-5}$, $p = 37{,}120$ gives

$$\delta\alpha = 7.1°$$

compared with 16.5° (rigid method); 31.7° (for $I_c = 0.1$); and 23.8° (for $I_c = 0.2$).

Conclusion

The results of these tests show that it is doubtful if Merton's simplified theory can be applied to an étalon. In the first test the differences between the value of $\delta\alpha$ found by the use of rigid theory and those obtained by the approximate method range from over 10 to nearly 100%. While it is possible that a set of conditions (that is, a more suitable p or I_c) might be found which would lead to satisfactory agreement, it seems certain that the approximate method cannot be used in the case of an étalon with plates 1 cm. apart.

In the second test the lack of agreement is even more pronounced. That this is so is scarcely surprising, for relation (12) is based on an assumption for which there is little justification. According to this relation the same value of $\delta\alpha$ is obtained, for a given p, for any value of r^2. Obviously this cannot be correct, since the distribution of intensity in the image is very markedly dependent on the reflecting power of the half-silvered surfaces.

We conclude then, that in order to apply Merton's method to an étalon, the complicated function given in relation (8) must be used in equation (7). The practical difficulty in thus obtaining k^2 from observed values of ϕ_c is enormous. On the other hand, if the problem consists (as is sometimes the case) in deciding whether the radiator of a line is the atom or the molecule, there are then only two possible values of k^2. In such a case the value of k^2 which satisfies equation (7) the better can be found by actual trial without an undue amount of labor.

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DURATION OF METASTABLE STATES[1]

By J. M. Anderson[2]

Abstract

Improved experimental methods for the measurement of time durations of metastable atoms have been developed, and measurements made for a series of pressures at room temperature and at liquid-air temperature of the decay curves for the absorption of λ 7635 Å in argon.

Introduction

The study of the duration of excited states whose life is limited and determined by collision phenomena offers one of the most promising avenues of approach to the general problem of velocity of reaction in gases, a problem which has hitherto been treated without any consideration of the energy level scheme of the molecules concerned. A preliminary attempt to construct a theory taking into account both the temperature distribution of molecular energies and the quantized energy states of the particles has been published by the author in a previous paper (1), so that any consideration of theory will be omitted from the present communication even though recently obtained experimental results allow this work to be considerably improved and extended. For an experimental attack on this problem the most suitable substances are the inert gases, because of the ease with which they can be brought to a high state of purity and because of the possibility of obtaining with them accurately known metastable levels in close proximity to a small number of non-metastable states. In addition, the duration of these states can be easily studied by the absorption of radiations which in emission arise from transitions to them from higher states. These radiations occur in regions easily accessible by photography.

Method

The study of the absorption produced by these states is a development from the pioneer work done by Meissner (2, 3) on this subject. In applying this method a long tube of pure gas is feebly excited by means of a continuous electrical discharge. With the gas excited in this way it is possible to obtain strong absorption of radiations arising from transitions to the metastable levels. The use of continuous discharge in the absorption tube yields no information as to the duration of the states but only gives a rough indication of the variation with pressure. In order to study the actual life-time it is necessary to excite the absorption tube by a short flash which cuts off sharply, and to project light from the emission tube through this gas in a short flash occurring at a known time after the excitation of the absorption tube has ceased. By varying this known time the decay of the absorption can be determined and from this we may hope to deduce information concerning the efficiency of collision phenomena.

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Contribution from the Physical Laboratory of the University of Toronto, Toronto, Canada.
[2] *Postgraduate student, University of Toronto, and holder of a fellowship under the National Research Council of Canada.*

Apparatus

Electrical Circuits

The problem reduces to that of producing short flashes in gas discharge tubes, whose intensity falls very sharply to zero at the end of the flash with no stray discharge following it. It is necessary that these flashes be produced at definite regular intervals so that the flashes through the absorption tube and through the emission tube can be separated by an accurately known time difference. Three different circuits were the subject of experiment.

I. The first circuit tried in this work was that used by a previous investigator (4) and is shown in Fig. 1. The transformer T was supplied with low voltage alternating current of the desired frequency. The secondary current, rectified by means of the valves V, passed through the resistance R and discharge tube D. The capacity C was shunted across the tube and resistance to cut down the duration of the flash through D. In spite of long and careful trials it was found impossible to make this circuit give satisfactory flashes with any combination of capacity and resistance available in this laboratory. The flash through the tube D, if the circuit were so adjusted that it cut off sharply, did not occur at the same phase in each cycle but jumped about by as much as 20°. On the other hand, when the resistance R was increased to swamp out variations in D so that the flash always came at the same part of the cycle, the flash itself was invariably followed by a stray discharge whose intensity fell off gradually from the last of the flash. If adjusted so that both of these faults were reasonably small, the circuit became so unstable that even a sudden jar was sufficient to change it into such a state as to exhibit one of these defects to a very serious degree. As a result of several months of tests this circuit was completely abandoned.

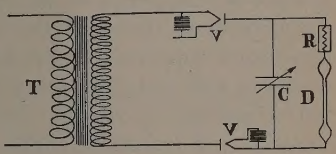


Fig. 1. *Excitation circuit I.*

II. Another circuit was developed whose principal features are shown in Fig. 2. The transformer T was supplied with alternating current as before. The secondary current passed through the synchronous switch S on the shaft of the generator of the primary current and so to the resistance R and tube D. The capacity C was placed across the transformer to take up the surges due to the presence of the switch S. This switch which was adjusted to be in phase with the secondary current consisted of two pointed rods insulated from the generator shaft by a bakelite disc. As these rotated they, at one phase of the cycle, came simultaneously opposite two fixed points so that the air gap in the circuit was small and the voltage could break it down. By the adjustment of the rotating points so as to be exactly on the same diameter it was possible to obtain two flashes in each cycle or by placing them some distance out of

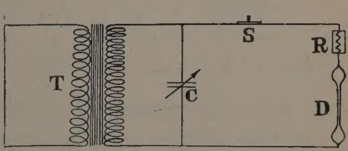


Fig. 2. *Excitation circuit II.*

line to obtain only one. This latter method was adopted for reasons concerned with circuit III (below). With this circuit perfectly regular flashes were obtained of a duration which was too short to be detected by any stroboscopic means available ($<10^{-5}$ sec.). There was never any trace of stray discharge or doubling of the flash in any of the tests of this circuit. An added advantage of this design was that the values of C and R could be varied within wide limits without seriously affecting the character of the discharge. In fact the chief reason for adding the resistance R was to give a convenient method of varying the intensity of the discharge. R was preferably a non-inductive resistance since it was found that the presence of a high inductance could be made to blur the flash slightly. Owing to the fact that the switch S did not make positive contact but depended on the breaking of an air gap to complete the circuit the excitation produced was rather high. This fault was sufficient to prohibit the use of this device in the emission tube circuit, for a Stark effect or other disturbance so distorted the lines that they were not absorbed even in an absorption tube excited by a direct-current discharge. So far as could be observed with the glass spectrographs used, the lines remained perfectly sharp but no absorption could be obtained with this type of source and no values of capacity and resistance eliminated this defect. This fault, however, did not preclude the use of this circuit for the absorption tube, as here the atoms are not called upon to absorb until after the flash (and the electric field) has ceased. The essential advantages of this circuit including its beautifully sharp regular flashes were just what were necessary in the absorption tube where the slightest trace of stray discharge following the flash would vitiate the results.

III. As circuit II proved unsatisfactory as a source after very exhaustive trials it was necessary to devise a third circuit. This circuit, which was a combination of the principles of I and II, is shown in Fig. 3. The secondary current was rectified by valves and passed through the resistance R and tube D as in circuit I. In order to make the flash occur regularly, however, a synchronous switch on the primary generator shaft was inserted. It was necessary that this switch close the circuit with a positive contact in order to give satisfactory results. A bakelite disc into which an annular brass ring was sunk was attached to the generator shaft. From this annular ring a small brass sector projected to the edge of the bakelite disc. As the whole rotated, a phosphor bronze spring pressed continually on the brass ring while another spring pressed against the disc outside the ring and thus made a positive metallic contact once in each cycle. This switch had to be adjusted to be accurately in phase with the natural breakdown point of the circuit or sparking occurred and the spectral excitation was too high for the work. This device effectively prevented the flash from moving its position in the cycle and the small size of the brass sector limited the time for which stray discharge could exist. This time however was very small, due to the other adjustments in the circuit.

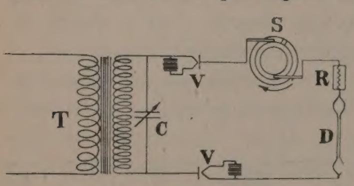


FIG. 3. *Excitation circuit III.*

Stray discharge also was obviously not so important in the emission tube so long as its intensity was reasonably small. The resistance R (non-inductive) had to be adjusted so that the circuit was not a free oscillator but it had also to be kept so small as to leave the intensity of the discharge high. Its value and the value of the capacity C were fixed therefore within rather narrow limits. The duration of the flash with this circuit was less than, but of the same order as, 10^{-4} sec.

In the final form of apparatus the absorption tube was run from a circuit of type II while the emission tube was run from a circuit of type III. Fig. 4 shows how these circuits were supplied with low voltage alternating current from two generators on a common shaft. Fig. 5 shows a detailed diagram of the variable coupling C which was used to set the relative phase of the armatures. The generators used were of a 50-cycle 750-watt 2-pole type with

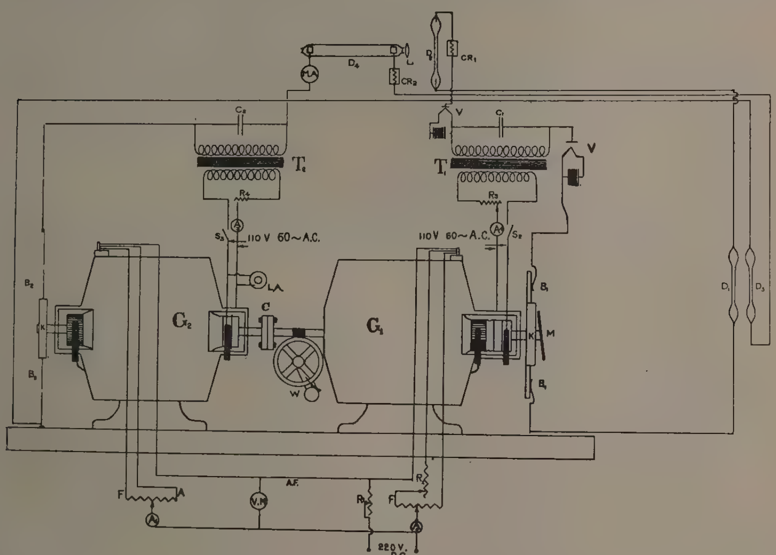


FIG. 4. *Complete electrical circuit.*

220 volts D.C. input and 110 volts output. In order to avoid damage due to surges which were sometimes rather violent when the circuit of type II was used, it was considered advisable to protect the generator by means of an ordinary telephone carbon block lightning arrester. This could be filed down to break at about 300 volts. The exact speed was measured by the worm gear and wheel W which rang a bell once every 250 revolutions. The speed was adjusted by a resistance R_1 in series with the input. The load was balanced between the generators by a field resistance R_2. The character of the flash was studied by viewing the image of tubes D_1 D_3 in the mirror M which also served as a check on phase difference. An even more sensitive method of studying the duration of the flashes and stray discharge was to view the polished

metal spikes B_2 by the light of $D_1 D_2$. Stray discharge which was quite invisible in the mirror was easily detectable by this method as a blurring of the outline of the spikes. Circuit II produced flashes so short that the images of the spikes, in so far as the eye could detect, were absolutely sharp throughout their entire length. It was found desirable to measure the current through the absorption tube by a thermocouple milliammeter directly in series with it, as the indication of the ammeter A_3 was not in the same proportion to it for all settings. The coupling C (Fig. 5) was calibrated in 400 equal divisions so that the time difference could be set (accurate to at least 0.5×10^{-4} sec.) at any time from 0 to 200×10^{-4} sec. In actual practice the time difference was never set at less than 3×10^{-4} sec. and this is taken as the arbitrary zero time of the curves below. This was done in order to avoid any possible chance of trouble due to stray discharge. For the same reasons settings were not made at time differences greater than 195×10^{-4} sec.

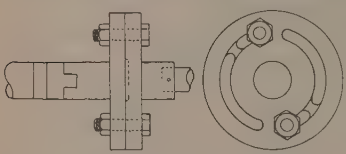


FIG. 5. *Adjustable coupling between generators.*

Discharge Tubes

As the most important part of the work was to be carried out at low temperature the absorption tube had to be so constructed that it could be kept surrounded by liquid air while the measurements were being made. Many designs were tried which differed in detail but the form finally adopted is that shown in Fig. 6. The discharge tube (Pyrex) was mounted upright in a vacuum flask as shown. The connection between the tube and flask at the lower end caused considerable difficulty. It was necessary to keep the liquid air from penetrating beneath the tube and so entering the light path. A ground glass joint was first tried but this device would not stand low temperatures. The joint finally adopted is a loosely-fitting glass sleeve packed with linen thread. Other materials tried as packing seemed to offer very little obstacle to the liquid gas. The electrodes were of coiled tungsten rod (50 mils). The use of tungsten avoided outgassing difficulties as the electrodes could be quite safely heated white hot and this effectively cleaned them. The structure of the glass-tungsten seals proved more rugged than was eventually found necessary. In the course of experiments with circuits of type II such heavy discharges were used in the absorption tube that other types of seal were quickly punctured. The cover for the flask was constructed of German silver to cut down heat conduction. In this a mirror was mounted as shown in Fig. 7. This reflected the light out through the side tube to the spectrograph. Gold sputtered mirrors were used because of their high reflecting power in the infra-red.

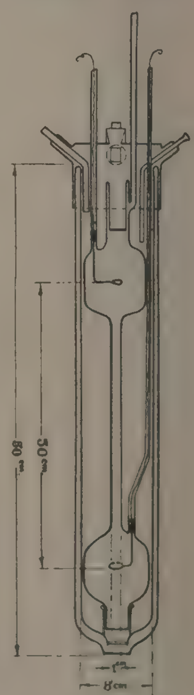


FIG. 6. *Low temperature absorption tube.*

The emission tube was an ordinary geissler tube of approximately 2-mm. bore, whose central tube was bent backward and forward several times in order to give a larger light area without increasing the depth of emitting gas. Tungsten electrodes were used here also. A reservoir of one-half-litre volume was connected to this tube to avoid any wide variations in pressure due to heating. This precaution was unnecessary in the final stages of the work as the discharge intensity was much less than that used during development.

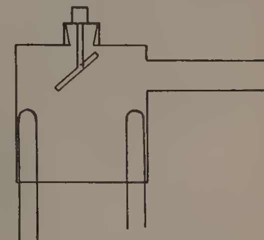


FIG. 7. *Cover for vacuum flask.*

The light passed from the emission tube, placed directly below the bottom of the flask, up through the absorption tube and so out to the spectrograph. It was found necessary to take the comparison spectrum at the same time as the absorbed spectrum by means of a system of mirrors taking light from the emission tube and bringing it to the spectrograph so that the absorbed and comparison spectra appear side by side on the plates. Unavoidable variations in the emission tube intensity demanded this precaution.

Experimental

After some preliminary work on helium with the first two excitation circuits described, which gave some rather uncertain results, it was decided to investigate argon even though this gas is more subject to disturbances in its spectrum

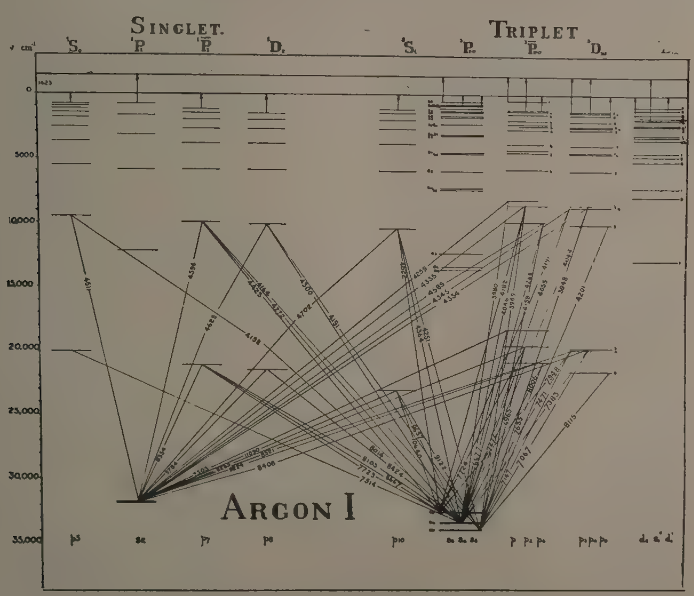


FIG. 8. *Energy level diagram.*

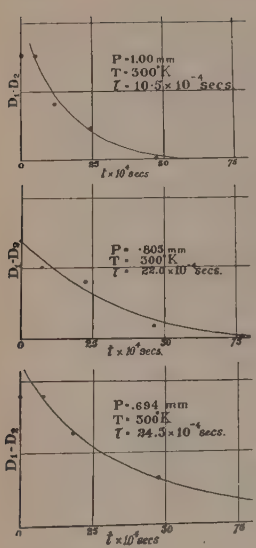


FIG. 9. *Decay curves of the absorption of λ 7635 Å.*

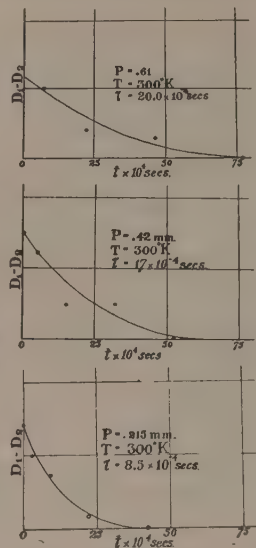


FIG. 10. *Decay curves of the absorption of λ 7635 Å.*

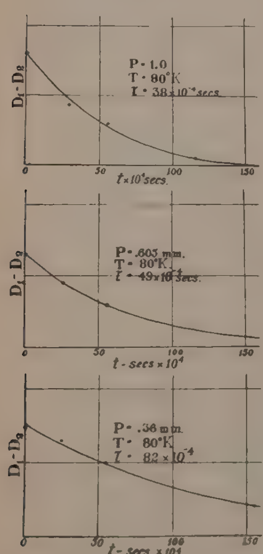


FIG. 11. *Decay curves of the absorption of λ 7635 Å.*

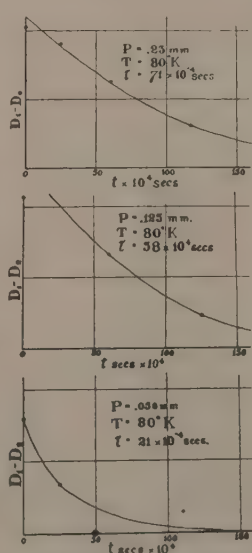


FIG. 12. *Decay curves of the absorption of λ 7635 Å.*

due to the high electric fields produced by circuit number II. This rendered it absolutely necessary to develop some circuit such as that of type number III. The argon was purified by passing a heavy arc between calcium electrodes in an atmosphere of the gas. This removed most of the impurities. A final purification was made just before the gas was admitted to the discharge tubes by passing a discharge between a potassium pool and a tungsten electrode in a bulb containing the gas. This method was very efficient and no trouble was experienced with impurities.

The energy level diagram for argon is shown in Fig. 8. As may be seen, almost the only isolated lines whose lower states are $^3P_0$ and $^3P_2$ are λ 7948 and λ 7635 respectively. The exposures were taken on a specially constructed high light power spectrograph with a direct vision prism and F 1.9 camera lens. The dispersion in the region around λ 7500 was about 150 Å per mm. The speed of this instrument is approximately 75 times that of a Hilger Constant Deviation glass spectrograph. For the preliminary work Neocyanin plates sensitized with ammonia were used. It was hoped that it would be possible to study λ 7948 with the same exposure as λ 7635, but this was found to be impracticable as the exposures necessary to bring out λ 7948 caused a considerable amount of fog on the background of the

plate. As the exposures showed that the total absorption of λ 7948 was in any case small in the absence of stray discharge, it was decided to confine the investigation for the present to λ 7635. This allowed the use of Kryptocyanin plates which, in addition to being much faster, require no pre-sensitization. The measurement of density of the plates was obtained from traces made with the Moll recording microphotometer. The curves shown in the accompanying figures indicate the relation between the value of $D_1 - D_2$ ($\alpha \log\frac{I}{I_0}$) and time differences. The reason for plotting this function against time instead of percentage absorption has been dealt with in the previous paper (1). The curves are all reduced to the arbitrary zero time mentioned above. The pressures used were measured on a McLeod gauge. Some uncertainty might arise about the true pressure at liquid-air temperature as the discharge vessel was at lower temperature than the gauge. This factor could not be checked very accurately, but by measurements on the same gas at the two temperatures it was shown that this error could not be very large.

Results

The curves shown in the accompanying figures (Fig. 9, 10, 11, 12, 13) indicate the decay of the function ($D_1 - D_2$) for λ 7635. As stated above very little absorption was observed for λ 7948. This indicates that the life of the $^3P_0$ state is short compared with that of the $^3P_2$ state. Such plates as were obtained indicated a half-life of approximately 2×10^{-4} for the $^3P_0$ state. It should be stated, however, that this value is very uncertain.

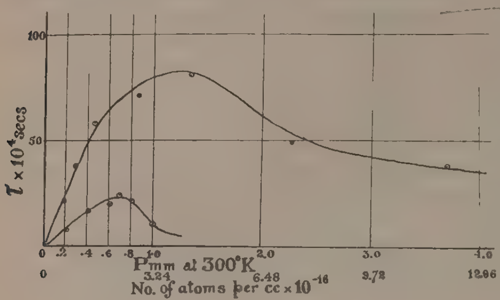
TABLE I

Data obtained from curves

P, mm.	N per cc.	τ, sec.
$T = 300°$ K		
0.215	0.697×10^{16}	8.5×10^{-4}
0.42	1.36×10^{16}	17.0×10^{-4}
0.61	1.97×10^{16}	20.0×10^{-4}
0.694	2.25×10^{16}	24.5×10^{-4}
0.805	2.60×10^{16}	22.0×10^{-4}
1.0	3.24×10^{16}	10.5×10^{-4}
$T = 80°$K		
0.050	0.607×10^{16}	21×10^{-4}
0.080	0.973×10^{16}	38×10^{-4}
0.125	1.52×10^{16}	58×10^{-4}
0.23	2.80×10^{16}	71×10^{-4}
0.36	4.37×10^{16}	82×10^{-4}
0.605	7.36×10^{16}	49×10^{-4}
1.0	12.15×10^{16}	38×10^{-4}

A few plates taken at liquid-air temperature indicate that even at this temperature the half-life of the state is small. In many of the curves shown the points at great time difference have been cut off so that the curves might be reproduced on a reasonable scale. The data given by the curves may be summarized in Table I, τ being the time in which the function (D_1-D_2) drops to one-half its initial value.

The first graph on Fig. 13 gives the master curves at the two temperatures. The maximum half-life at room temperature, it will be seen, fell at $N=2.85\times10^{16}$ per cc. On the other hand, the maximum half-life at liquid-air temperatures fell at $N=4.08\times10^{16}$ per cc. The general shape of the master curves corresponds quite well with theoretical predictions (1).



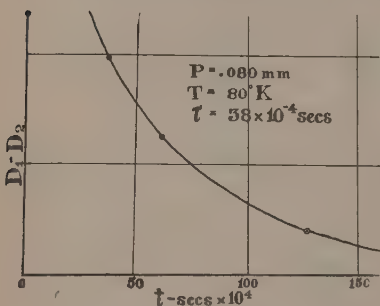


FIG. 13. *Master curves of half-life and decay curve.*

Conclusion

The accuracy of individual values of the function (D_1-D_2) as plotted is about 10%. Two completely independent plottings of the same points gave half-life values which agreed for the two sets very well. The maximum difference obtained between the value assigned to a given half-life in the two sets was 4%. The internal agreement of the values as shown by the master curves would also indicate that the accuracy was at least as good as this percentage. The measurements were not made in progression from high to low pressures or the reverse. The pressures used were studied in random order with the intention of eliminating any progressive change in the apparatus. It was considered that any effects arising from stray discharge could be safely neglected as the character of the flashes used had been made the subject of exhaustive study and the absorption tube circuit never under any circumstances showed any trace of this defect that could be detected by the most delicate methods available.

Acknowledgments

The author wishes to express his thanks to Professor J. C. McLennan, who supervised the work, and also to the members of the workshop staff of this laboratory, especially to Mr. Plaskett and Mr. Woodward, without whose advice and assistance the work would have been seriously hampered. To Mr. Chappell the author owes his thanks for the construction of the discharge tubes.

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THE DIELECTRIC CONSTANTS OF HYDROGEN PEROXIDE-ETHER AND HYDROGEN PEROXIDE-WATER-ETHER MIXTURES[1]

By E. P. Linton[2] and O. Maass[3]

Abstract

The dielectric constants of solutions of hydrogen peroxide and of hydrogen peroxide-water mixtures in ether have been determined over a wide range of concentrations. It was shown: (a) that the dielectric constant of hydrogen peroxide in ether is proportional to the amount of hydrogen peroxide per unit volume, and (b) that the variation of the dielectric constant with mole fraction was proportional to the dielectric constant of the solution examined, so that the logarithm of the dielectric constant varies in a linear way with the molecular fraction. By this means the dielectric constant of pure hydrogen peroxide at 0° C. was found to be 93.7. It has been shown that hydrogen peroxide-water mixture has a higher dielectric constant than either constituent. The densities of ether-hydrogen peroxide solutions were measured and a maximum aberration from the mixture rule found at a 1:1 concentration.

Introduction

The dielectric constant of hydrogen peroxide has been determined in this laboratory (2) by a step-by-step method based on air in the first step, followed by the use of benzene, ether, nitrobenzene and water in the intermediate steps. The method has been reinvestigated and several corrective factors have resulted in new values being assigned to the dielectric constants involved. These results have been published elsewhere (3). As a result, the recalculated value of hydrogen peroxide was found to be 93.7 and that of its aqueous solutions were changed in a corresponding way.

In this paper the dielectric constant of hydrogen peroxide is obtained by the extrapolation of the dielectric constants of ether solutions of varying concentrations. This is of particular interest, since such solutions are far less conducting and decomposition of the hydrogen peroxide on the metal of the dielectric cell is much less than in aqueous solutions. Furthermore the former determination (2) of the dielectric constants of aqueous solutions of hydrogen peroxide showed an unexpected maximum, the existence of which was also investigated by means of ether solutions.

Measurement of Dielectric Constant

The apparatus used was similar to that described by Cuthbertson and Maass (1). A diagram of the connections is shown in Fig. 1. *A* is the dielectric cell containing the liquid whose dielectric constant is to be measured. *B* is a variable cell connected in parallel with the inductance and dielectric

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cell. The accuracy of the method depends to a large extent on the construction of this tuning condenser. It should give a straight-line relationship between capacity and dial setting in the region used. This was found to be the case over the range of the condenser used in these experiments. A glass mirror was mounted on the tuning condenser and the setting read on a four-foot circular scale by means of a reflected beam of light. A condenser *C* was kept in series with the tuning condenser. This gave a much stronger note when measuring the dielectric constant of a liquid like water. A *UX*-171-*A* radiotron tube *F* was used as the source of oscillations. This required 90 volts in the plate circuit and 6 volts on the filament. A choke coil *G* was placed in the plate circuit to absorb the radio-frequency oscillations and prevent them passing through the plate circuit. Fig. 2 shows a fixed oscillator operating on a fixed frequency throughout any measurement. The ordinary Hartley hook-up was used. A *UX*-199 radiotron tube, *C*, taking $22\frac{1}{2}$ volts on the plate and 3.5 volts on the filament, was used. A number of cells in parallel were used as the source of current in order to keep the voltage as constant as possible during any one measurement. The telephones *E* indicated the point of resonance, *i.e.*, when the two oscillators were operating on the same frequency. The dielectric cell, *A*, was made of pure block tin. It was constructed on the principle of a variable condenser, the plates of which were to be immersed in the liquid whose dielectric constant was to be measured. The rotor plates were attached by means of an insulating bakelite board to the stator plates. By means of a vernier dial attached to the rotors, the variable condenser could be set at any desired capacity. This cell was supported in a Pyrex glass beaker which contained the liquid under measurement. This beaker was surrounded by a larger beaker containing ice and water by means of which the liquid under measurement was kept at a constant temperature. This method had the advantage of eliminating end effects and the necessity of correcting for the capacity of the leads.

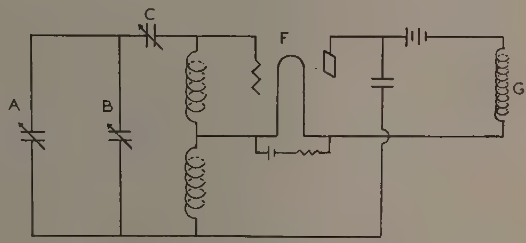

FIG. 1. *Diagram of connections.*

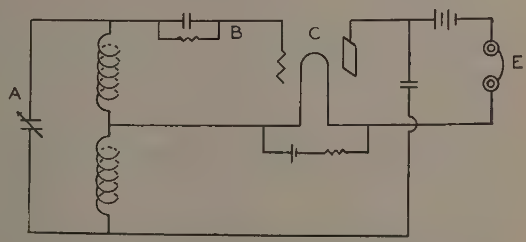

FIG. 2. *Diagram showing a fixed oscillator operating on a fixed frequency throughout any measurement.*

The dielectric cell was set at a definite reading and the variable oscillator brought into resonance by means of the tuning condenser. This process was repeated for a number of settings of the dielectric cell. In this way a straight-line relationship was obtained between scale readings of the tuning condenser and settings of the dielectric cell. The ratio of the slopes for the two media in the dielectric cell was proportional to the ratio of their dielectric constants, since the plates of the cell occupied the same position at corresponding settings.

Since the dielectric constant of ether is around four and that of hydrogen peroxide over 90, dielectric cells of different capacity had to be used for the determinations of the dielectric constants of mixtures of the two liquids. A three-plate dielectric cell was used to measure the dielectric constant of solutions between 30 and 60% hydrogen peroxide. The dielectric constants of these strong solutions could be measured directly by comparing their slopes with those for pure water or nitrobenzene.

For the more dilute solutions a seven-plate condenser was constructed and a mixture of nitrobenzene and benzene was used for comparison, this solution being calibrated against both ether and nitrobenzene. Very dilute solutions were compared directly with pure ether. To prevent, as much as possible, the evaporation of ether from the solution, a sheet of rubber was glued to the bakelite cover of the dielectric cell. This rubber fitted tightly over the top of the beaker and served at the same time to keep the moisture of the air out of the solution.

All these measurements were carried out on a wave-length of 250 to 300 metres.

Preparation and Analysis of Solutions

The anhydrous peroxide was prepared in the usual way (4). A 99.4% product served in these experiments since the small fraction of water, whose dielectric constant is about the same, had no appreciable effect. For these measurements, ordinary commercial ether was shaken with water five or six times, and the water layer discarded. The ether was then dried over calcium chloride, distilled and allowed to stand over sodium wire until no more bubbles were given off. Any trace of sodium oxide or sodium hydroxide in the ether would cause the hydrogen peroxide to decompose. To avoid this the ether was distilled from fresh sodium in a moisture-free Pyrex still.

Ether dissolves hydrogen peroxide up to a concentration of about 60% by weight, with evolution of heat. The composition of the solution could be determined by analyzing for hydrogen peroxide with standard potassium permanganate or by making the solution up to a known composition by weighing the hydrogen peroxide and ether separately. In the more concentrated hydrogen peroxide solutions no difficulty was found in obtaining an end-point with potassium permanganate, but when the concentration of ether was over 70%, it was difficult to obtain the correct end-point. The excess ether had to be evaporated off before diluting with water. If a large excess of water, *e.g.*, about one and a half litres, was used, the end-point could be obtained more easily.

The solutions were made up to the strength desired by mixing together known weights of hydrogen peroxide and ether. The first solution made up was the most concentrated that could be obtained. This was diluted with known weights of ether and the more dilute solutions were obtained in this way. The strength of the solutions was then checked by analyses and in all cases were found to have a concentration agreeing with that calculated from the amounts mixed together.

Any inorganic impurities dissolved in the hydrogen peroxide or ether do not ionize to any extent in the solution. Hence the conductivity of the solution is low and will not effect the measurement of the dielectric constant.

Difficulty was encountered in obtaining an ether solution of a mixture of 50% water and 50% hydrogen peroxide. Only a 3% solution could be obtained, a second phase appearing at about 5%. A mixture consisting of 87% hydrogen peroxide and 13% water dissolved quite readily in ether up to a concentration of 50%. The dielectric constants of these solutions with various dilutions with ether were measured. The concentration of the different constituents was determined by weighing the amounts added to make up the solution.

Density of Solutions

The addition of hydrogen peroxide to ether is accompanied by the evolution of heat and a volume change. Since a knowledge of the latter is necessary in the calculation of the molecular concentration, the densities of the ether solutions were determined.

These were measured by means of a dilatometer with a graduated stem. A tight-fitting glass cap was made to cover the end of the tube, so as to avoid as much as possible the loss of any ether by evaporation. The concentrations of the solutions were determined, as previously described and measurements were carried out at 0° C. The usual corrections were made to reduce the weights to vacuum.

Experimental Results

The results obtained have been condensed and are shown in Tables I and II. The first column in these tables gives the percentage by weight of solute in ether as solvent.

TABLE I

Dielectric constants of hydrogen peroxide-ether mixtures

H_2O_2, % by weight in ether as solvent	Density	Dielectric constant
0	0.736	4.6
5.2	0.769	6.7
10.0	0.791	8.7
17.8	0.834	13.2
26.3	0.892	18.0
38.2	0.954	26.9
46.3	0.997	33.8
58.2	1.071	44.3

Note:—*Solute consists of pure hydrogen peroxide.*

TABLE II

Dielectric constants of a solution of a mixture consisting of 87% hydrogen peroxide and 13% water, in ether

$H_2O_2-H_2O$ mixture, % by weight in ether as solvent	0	10.6	25.3	38.0	49.3
Density	0.736	0.792	0.872	0.944	1.005
Dielectric constant	4.6	8.7	18.7	28.8	41.2

Discussion of Results

Inspection of the variation of density with composition shows that a decrease in volume occurs. This is shown in curve 1, Fig. 3, where density is plotted against molecular fraction of hydrogen peroxide.

The aberration from the mixture rule can be calculated from the following formula,

$$V = 1 - \frac{\left(\frac{\%\ H_2O_2}{100}\right)D_1}{D_2} + \frac{\left(1 - \frac{\%\ H_2O_2}{100}\right)D_1}{D_3}$$

where V is the aberration in 1 cc., D_1 is the density of the solution, D_2 is the density of pure hydrogen peroxide at 0° C. (1.465), and D_3 is the density of ether at 0° C. (0.736).

Table III gives the percentage composition, mole fraction and aberration from the mixture rule of the various solutions examined.

TABLE III

Composition and aberration from the mixture rule

H_2O_2, %	Mole fraction	Aberration
5.2	0.1022	−0.029
10.0	0.195	−0.032
17.8	0.323	−0.042
26.3	0.436	−0.064
38.2	0.574	−0.061
46.3	0.654	−0.051
58.2	0.750	−0.042

These points are represented in curve 2 of Fig. 3. A maximum aberration occurs at a density of about 0.93 which corresponds to a mole fraction of 0.5 H_2O_2. This maximum occurring at a 1:1 molecular mixture might be taken to indicate that a compound of ether and hydrogen peroxide was formed. On the other hand the maximum deviation at this point may be fortuitous. When the dielectric constant of the hydrogen peroxide-ether mixture is plotted

against percentage composition a smooth curve is obtained. Due to the high molecular weight of ether relative to that of hydrogen peroxide, the curvature is increased by plotting mole fractions as abscissas. Finally a straight-line relationship is obtained when the number of grams of hydrogen peroxide per unit volume of solution are plotted as abscissas. These values are tabulated below together with other units which are used in obtaining straight-line relationships.

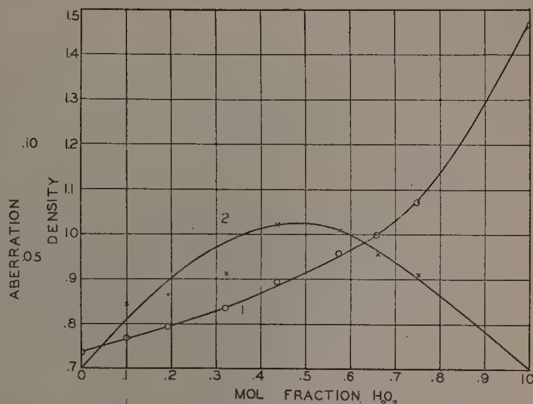

FIG. 3. *Curve 1. Variation of density with mole fraction hydrogen peroxide. Curve 2. Variation of the aberration from the mixture rule with mole fraction hydrogen peroxide.*

TABLE IV

VALUES USED IN OBTAINING STRAIGHT-LINE RELATIONSHIPS SHOWN IN FIG. 4 AND 5.

Mole fraction	Gm. H_2O_2 per unit volume	E	Log E
0	0.000	4.6	0.662
0.1022	.040	6.7	0.826
0.195	.079	8.7	0.939
0.323	.148	13.2	1.12
0.436	.234	18.0	1.25
0.574	.364	26.9	1.43
0.654	.461	33.8	1.52
0.750	.623	44.3	1.64
1.000	1.464		

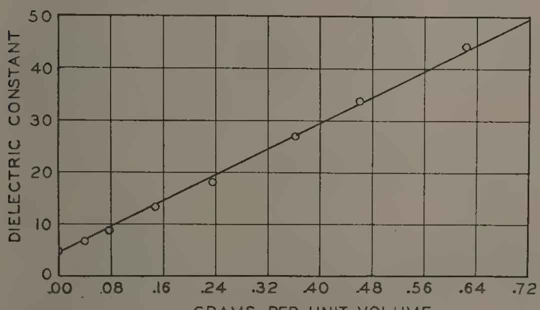

FIG. 4. *Straight-line relationship between dielectric constant and grams per unit volume.*

In Fig. 4 grams hydrogen peroxide per unit volume are plotted against the dielectric constants of the corresponding solutions. Within the limits of experimental error a straight-line relationship is obtained which can be represented by

$$E=60.8\ (x)+4.6$$

where E is the dielectric constant of a solution containing x gm. of hydrogen peroxide per cc. The fact that a straight-line relationship is obtained can be interpreted as meaning that the dielectric constant of a solution is proportional

to the amount of hydrogen peroxide in an inert medium having a dielectric constant of 4.6. By inert is meant that the medium apparently does not in any way change the dielectric constant of the hydrogen peroxide. This goes to show that the maximum volume aberration at a 1:1 molecular mixture of ether and hydrogen peroxide, dependent on a molecular compound formation, can only be due to the formation of a minute amount of such a compound. Hydrogen peroxide in ether therefore behaves in a very different manner from hydrogen peroxide in water, where no such straight-line relationship is obtained.

From the equation it is possible to extrapolate to the dielectric constant of pure hydrogen peroxide. At 0° C. the density of pure hydrogen peroxide is 1.46. By inserting the value of 1.46 gm. per unit volume the dielectric constant is found to be 93.4, which is in good agreement with 93.7 obtained by direct measurement.

The above extrapolation is over a large distance due to the peculiar units used. By chance another straight-line extrapolation was arrived at, which can be carried out graphically because, on the basis of the units used, the experimental points extend for more than three-quarters of the length of the complete curve after extrapolation.

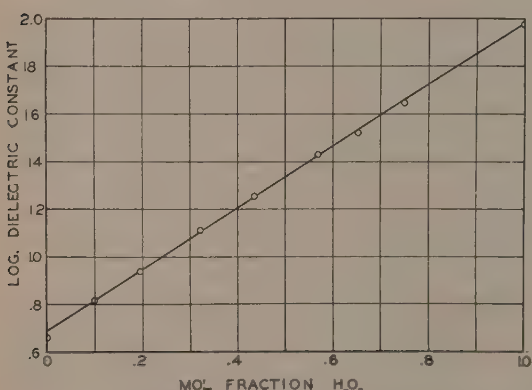

FIG. 5. *Extrapolation of the dielectric constant of hydrogen peroxide-ether solutions.*

The relationship depends on the variation of the dielectric constant of the solution with the mole fraction of peroxide being proportional to the dielectric constant of the solution, that is $\frac{dE}{dy} = KE$, where the variables E and y are dielectric constants and mole fractions respectively. On integration this gives $\log E = Ky + C$. In Fig. 5 the relation between $\log E$ and y is shown to be a straight line, the values being taken from Table IV. The value of E at $y = 1$, obtained from the graph, is 93.7, which is identical with that obtained by direct measurement.

TABLE V

CALCULATION OF MOLE FRACTION AND LOG E USING DATA IN TABLE II

Mole fraction $H_2O_2-H_2O$	0	0.220	0.447	0.603	0.703
Dielectric constant	4.6	8.7	18.7	28.8	41.2
Log E	0.66	0.939	1.27	1.46	1.61

The dielectric constants of solutions of water and hydrogen peroxide are considerably greater over a large part of the concentration range than the value for either substituent.

The data of Table II were used for the calculation of mole fraction and log E (Table V).

In Fig. 6 the graphic extrapolation gives a value of 107 for the dielectric constant of a mixture of 87% hydrogen peroxide and 13% water. The value for the water solution obtained by Cuthbertson and Maass gives on recalculation a value of 103. This difference is to be expected since the experimental error both in the hydrogen peroxide-water direct determination and in the three-component systems is much larger. Furthermore if the increase in the dielectric constant of hydrogen peroxide-water mixtures is due to a complex formation this would be more complete in an ether solution and consequently it would lead to a higher value in this case.

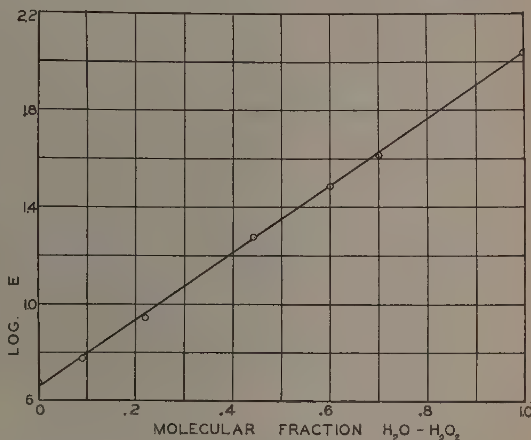

FIG. 6. *Extrapolation of the dielectric constant of ether-water-hydrogen peroxide mixtures.*

The resonance method for the determination of the dielectric constant of hydrogen peroxide and water has been criticized from the point of view that the influence of the conductivity of such solutions would have an influence on the experimental observations. Reference has been already made to an investigation (3) in which it was shown that this criticism is not justified. The results obtained above made in a non-conducting medium give extrapolated values agreeing with those found directly. Therefore apart from the interest attached to data obtained and their interpretation, this investigation may be looked upon as additional evidence for the validity of the resonance method.

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TRIATOMIC HYDROGEN[1]

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Abstract

A study was made of the activation of hydrogen in the alternating-current corona discharge at atmospheric pressure. The resulting test for sulphide was negative unless the gas contained a small percentage of oxygen. Oxygen promoted the activation by acting as a wall poison. Stearic acid also acted as a wall poison in a vacuum tube discharge through hydrogen at pressures from 20 to 80 mm. The hydrogen was freed from oxygen by passing gas from a compressed cylinder over platinized asbestos heated to 550° C. A fatigue effect appeared which was found to be due to the oxygen content of the hydrogen and to the condition of the walls of the discharge tube.

Quantitative determinations of the relation between concentration of the active gas and the velocity of hydrogen show that, at 42 mm. pressure, the concentration varied directly with the velocity up to an optimum flow of 2.4 litres per hour. At a higher rate the concentration varied inversely with the velocity. The per cent activation was found to vary inversely with the pressure between 30 and 80 mm. In the corona discharge the maximum per cent activation for hydrogen containing 0.1% of oxygen was 0.001.

A concentration of 0.025% active hydrogen was found for the vacuum discharge in a tube the walls of which were poisoned with stearic acid, and in which the pressure and velocity were 42 mm. and 2.4 litres, respectively.

Introduction

Numerous investigators* have reported the formation of an active variety of hydrogen in discharge tubes at pressures ranging from 2 cm. to atmospheric. The types of discharge used have been alternating-current corona, d.-c. corona, vacuum, and ozonizer.

J. J. Thomson was the first to show that the group H_3^+ existed when a discharge was passed through hydrogen at reduced pressures. Since this pioneer work was completed, Dempster (7), Hogness and Lunn (10), Smyth and Brasefield (23), have verified the existence of H_3^+. The latter workers have also shown that in a discharge through hydrogen at 0.14 mm. pressure, the ions of one positive charge are practically all H_3^+. At lower pressures the proportion of H^+ and H_2^+ increases while that for H_3^+ decreases. Wendt and Landauer (27) have observed that during a discharge through hydrogen in a closed system, the pressure decreases. Their evidence for the formation of a neutral triatomic molecule is based upon these contraction experiments. The name triatomic hydrogen is used for this active gas to distinguish it from Langmuir's (12) atomic hydrogen formed on high temperature filaments in evacuated bulbs and Wood's (29-32) atomic hydrogen produced in high tension discharge tubes at very low pressures.

Triatomic hydrogen is considered to show special chemical activity toward flowers of sulphur, forming hydrogen sulphide which reacts to produce lead

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**See References 1, 2, 8, 9, 14, 18, 25, 27.*

sulphide when the gas stream is directed against paper moistened with lead acetate.

Paneth, Klever, and Peters (19), Urey and Smallwood (22), and others have studied various methods of producing the triatomic gas but report any activity shown as being due to contamination of the reaction chamber with sulphur.

In the production of atomic hydrogen, Wood (29-32) used electrolytic gas containing both oxygen and water vapor; with pure dry hydrogen the atomic gas was not obtained. He considers that the water vapor poisoned the glass walls of the discharge tube and prevented the catalytic reversion of atomic to molecular hydrogen. He also shows that a tungsten wire will promote the reversion.

Bonhoeffer (5) in a study of the catalytic effect of metals upon the reversion of atomic hydrogen finds that copper, lead, bismuth, silver and mercury promote the action, while aluminium, magnesium, chromium, cobalt, nickel, and zinc, are without influence. Von Wartenberg and Shultze (26) have shown that ortho and meta phosphoric acid may be used successfully in a discharge tube to retard the reversion by acting as a poison on the glass walls.

Lewis (13) has shown that a small amount of water vapor or paraffin will poison the walls of a tube and increase the life of the glow in active nitrogen.

In the study made by Anderegg and Herr (1) the most marked fatigue effects were observed in the formation of active hydrogen when they operated the corona discharge tube filled with glass wool.

In view of the fact that the catalytic influence of glass surface of the discharge tube plays a very important role in the production of atomic hydrogen and glowing nitrogen, a study of glass surface effect might show some reason for the discrepancies existing regarding the production of triatomic hydrogen. The vacuum tube alternating-current discharge and the alternating corona discharge were selected since they were the types originally used by Wendt and Landauer, Anderegg and Herr, and Urey and Smallwood.

Experimental

Apparatus

The apparatus used in this work was made from ordinary glass. A Leibig condenser 65 cm. long having a central tube 1.25 cm. in diameter was used as a corona tube. The high tension electrode was a No. 26 B. and S. platinum wire fixed to a lead-glass post sealed in the exit. The opposite end was sealed into the central tube. The region occupied by the discharge tube was 45 cm. long. The ground electrode was a dilute solution of sulphuric acid circulated through the jacket by means of an air lift.

The energy was obtained from a rotary converter and the power output was regulated by an automatic field resistance. The alternating current was connected through a water rheostat to the primary of a Thordarson 25,000-volt 1 kva. Type R transformer. The secondary of the transformer was connected to the central electrode of the corona tube and the resulting discharge appeared very uniform along the central wire. The operating potential of the tube was about 12 kv.

The vacuum tube was blown from ordinary 6-mm. glass tubing and shaped in the form of a U-tube to permit immersion in an ice-water bath. Two 14-mm. tubes were sealed to the ends of the U-tube, the left being connected to the incoming gas line by a T-seal, while the one on the right was connected to the reagent tube. Electrodes of No. 15 B. and S. platinum wire were sealed into two smaller glass tubes which were connected to the 14-mm. tubes by inner seals. During all runs this tube was immersed in an ice-water bath to a point above the electrodes. This tube proved very substantial during almost continuous daily operation.

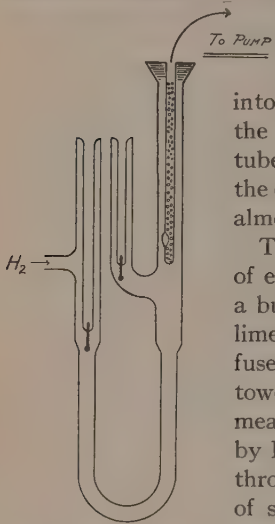

FIG. 1. *Vacuum tube.*

The hydrogen used was from a commercial cylinder of electrolytic gas. It was purified by passing through a bubbler containing concentrated sulphuric acid, a soda lime tower, platinized asbestos furnace, two towers of fused sodium and potassium hydroxide, and finally, a tower of phosphorus pentoxide. The gas velocity was measured by a calibrated differential flowmeter described by Benton (4). After the gas left the discharge it passed through, at 1.5 cm. distance, a tube containing flowers of sulphur well mixed with glass wool. It was then pumped to a small bottle where it was directed against a filter paper dipping in lead acetate solution. The bottle was connected through a drying tower to a Cenco Hyvac pump. Pressures were read from a manometer attached to the line near the discharge tube. Any activity was detected by the formation of lead sulphide on the filter paper.

Blanks

A careful study of the technique used by various workers in this field, emphasizes the importance of running a proper system of blanks. It is extremely difficult to operate a discharge tube at less than atmospheric pressure with flowers of sulphur in the line and have no sulphur blow back into the discharge. In some of the work previously reported, the criticism has been that the positive results are influenced by sulphur blown back into the discharge.

In this work a perfect blank constituted running the hydrogen from the purifying system through the tube with the discharge off and over sulphur without producing a trace of hydrogen sulphide, as shown by blank test paper. The sulphur tube was then removed, the discharge turned on, and the gas directed against lead acetate paper, producing no lead sulphide.

The first part of the blank showed that the gas was free from hydrogen sulphide, that it contained no constituent which reacted with sulphur, and that the sulphur contained no volatile sulphides. The second part of the blank showed that the hydrogen gas contained no sulphur compound capable of forming hydrogen sulphide in the discharge, and that the tube was free from contamination. The second portion of this test caused most trouble since

in a one-hour run, there was always obtained a trace of lead sulphide where a blank was expected. It was believed that the bubbler containing concentrated sulphuric acid was introducing a sulphur compound. After removing this and introducing another drying tower, the blanks showed no trace of sulphide after several hours continuous operation.

Since this investigation was completed, Truesdale (24) has reported a similar contamination. Throughout this work a great many blanks were run, especially when flowers of sulphur was used. It was found, at times, that the discharge tube was contaminated with sulphur. However, after washing with carbon tetrachloride, alcohol, and ether, the tube was free from contamination.

Preliminary Experiments

Urey and Smallwood (22) reporting an attempt to duplicate the work of Wendt and Landauer, state, "During the whole series of experiments sporadic tests were obtained but these were so small that it was necessary to run for 30 min. or more in order to produce a visible amount of lead sulphide." From these results, they concluded that triatomic hydrogen is not formed to an appreciable extent in the above types of discharge.

However, in their work the activated hydrogen from the discharge was led through 10 cm. of glass wool before coming in contact with sulphur. A glass-wool filter one inch in length was used by Wendt and Landauer. Perman and Graves (20) have shown that the rate of decomposition of ozone is largely dependent upon the condition and amount of glass surface. An increase of six times in the area increased the rate of decomposition sixtyfold; contact with glass wool increased the rate 127 times. If contact with glass wool influences the rate of decomposition of ozone so greatly, it is rather remarkable that any triatomic hydrogen should survive 10 cm. of glass wool.

During the early part of this investigation, the results were positive, in contradiction to the report of Urey and Smallwood. During 0.5-hr. runs considerable quantities of lead sulphide were obtained. After making runs over a period of several days, it was noticed that a fatigue effect appeared toward the end of the day; smaller amounts of lead sulphide were obtained. It was suspected that the tube was contaminated, but the tests were blank. At the beginning of each day, the amount of active gas formed was as much as on the previous day; the fatigue effect had disappeared. As it was thought that the walls of the tube had adsorbed some hydrogen sulphide, the air of the room was aspirated for two days in contact with lead acetate paper but no sulphide appeared. Other investigators (1, 22, 27) have noticed this fatigue effect. Anderegg's experience with the recovery of a tube from fatigue on standing was the same as the writers', who found that a tube showing maximum fatigue could be restored to normal by filling it with air and operating the discharge for 15 min. The use of nitrogen or water vapor proved unsuccessful; possibly the quantities were too large. Hydrogen, direct from the cylinder of compressed gas and containing oxygen and a trace of moisture, was then tried and no fatigue effect was noticed. Anderegg and Herr tried 1 part in 10,000, or 0.01% of oxygen mixed with the hydrogen, but reported no effect on the yield of active hydrogen.

The fatigue effect in the tubes used in this investigation was considered to be due to the disappearance of oxygen and water vapor from the glass walls during the electronic bombardment. During a day's run, the discharge tube would be open to the atmosphere very little. Very dry hydrogen streaming through the tube all day, with the discharge operating at regular intervals, would tend to remove from the glass walls the adsorbed oxygen and water vapor. Anderegg and Herr observed the most marked fatigue effects when the discharge tube was filled with glass wool.

It was considered that for the velocities of hydrogen used, 2 to 15 litres per hour, the platinized asbestos furnace was not at a high enough temperature to remove all the oxygen. Accordingly, the temperature of the furnace was raised to about 550° C. This had a marked effect upon the duplication of previous runs. The tube showed fatigue in a very short time. The writers were able to obtain perfect blanks with the glass-wool filter removed and the escaping gas passing over sulphur at 1.5 cm. from the discharge.

Urey and Smallwood point out that an unknown catalytic effect may account for their sporadic tests and the uncertainty of their results. This idea is also mentioned by Anderegg and Herr in referring to the work of R. W. Wood (29-32).

Mitchell and Marshall (16) after repeating the work of Anderson (2) report that a trace of oxygen in the hydrogen is necessary to explain the activation effect. Proper blanks were run and their conclusion was that small amounts of triatomic hydrogen were formed.

Bonhoeffer and Kaminsky (6) in studying the influence of traces of foreign gases upon the after-glow in active nitrogen, found optimum glow effects at the following concentrations: hydrogen sulphide, 0.04; methane, 0.1; oxygen, 0.23%. Less than 0.03% had no effect, with 0.5% no after-glow was observed and the optimum effect was produced with about 0.2% of oxygen present.

The abundance of experimental evidence relating the stability of an activated gas to the condition of the walls of the container shows that in any study of metastable constituents formed in a discharge tube, the wall condition should be considered as a factor influencing the results. The writers' initial experiments confirm this.

Further Experimental Work with Poisoned Walls

Corona Tube

The subsequent experiments were conducted with the walls of the discharge tubes covered with oxygen and water vapor (from the oxygen admitted), paraffin, and stearic acid. The corona tube was operated at atmospheric pressure with hydrogen mixed with varying concentrations of oxygen. The hydrogen was purified as previously described; the oxygen, by passing it through a strong solution of sodium hydroxide, drying towers of soda lime and calcium chloride, a platinized asbestos furnace, towers of fused potassium and sodium hydroxides, and finally, a tower of phosphorus pentoxide. The velocity of each gas was regulated through calibrated flowmeters. From these, the gases passed to a mixing chamber and then to the discharge tube.

Proper blanks were run with hydrogen and oxygen mixtures. The concentration of oxygen was varied from traces to concentrations high enough to form considerable moisture and alter the character of the discharge. No appearance of sulphide was noticed during these tests for periods of 1.5 hr.

The sulphur tube was then placed in the line (2 cm. from the discharge) and the runs repeated for one-hour periods without a glass-wool filter. Traces of oxygen, mixed with hydrogen, passing through the discharge gave no sulphide, but as the concentration of oxygen was increased to about 0.1%, increasing quantities of sulphide appeared on the test paper (Fig. 2 and 3).

In Fig. 2 are shown some of the papers used in the test for sulphide in the preliminary work. The duration of each run was one hour with a velocity of hydrogen at 10 litres per hour. No record was made of the oxygen rate but the concentration was low; it was varied from too large a quantity to smaller concentrations. The sulphur tube was 2 cm. from the discharge.

Fig. 3 shows one of the series of sulphide papers made during 0.5-hr runs by the a.-c. corona discharge using plastic sulphur as a testing agent placed 4 cm. from the discharge. During all runs the hydrogen velocity was 12 litres per hour.

In considering the amount of sulphide formed from the intensity shown it appears that No. 146, 147, 148, 152, show a maximum at 0.1% of oxygen; No. 144, 151, and 153 show a small amount of sulphide. It seems that No. 144 has too little oxygen added and No. 151 and 153 have too high a percentage of oxygen in the mixture. The blanks show the gas stream free from sulphur and the tube not contaminated.

Experiments with Hydrogen Containing a Small Percentage of Oxygen in Vacuum Discharge

Runs were made with vacuum discharge through a gas mixture of approximately 0.1% oxygen in hydrogen over a pressure range of 20 to 80 mm. for periods of 0.5 hr. The blanks run on these mixtures showed no sulphide formation. Table I shows the data from a few of the numerous runs.

TABLE I

THE EFFECT OF OXYGEN UPON THE FORMATION OF ACTIVE HYDROGEN

No.	Pressure in mm. Hg.	Distance of sulphur from discharge	Sulphide
96	36	No sulphur	None
97	20	2.5 cm.	Heavy
98	25	No sulphur	Slight
99	20	No sulphur	None
100	21	2.5 cm.	Heavy
101	20	No sulphur	None

NOTE:—*Small glass-wool filter used; oxygen, 0.1%.*

In run No. 98, there was a slight amount of sulphide formed, probably due to hydrogen sulphide (from run No. 97) adsorbed on the walls of the tubing

between the sulphur tube and the test bottle. This did not appear when the line was thoroughly flushed by pumping gas through after the discharge was turned off; run No. 101 shows this.

Where a tube had been contaminated by sulphur blown back a heavy sulphide test was shown for a good many runs. Often the surest method was to take the tube out of the system and wash it with carbon tetrachloride, alcohol and ether.

Experiments with Vacuum Tube Coated with Paraffin

TABLE II

THE EFFECT OF PARAFFIN COATED WALLS UPON THE FORMATION OF ACTIVE HYDROGEN

No.	Pressure in mm. Hg.	Distance of sulphur from discharge	Velocity of hydrogen in litres per hr.	Sulphide
106	35	3 cm.	3.55	Heavy
107	35	No sulphur	3.40	Slight
108	28	No sulphur	5.15	Slight
109	28	3 cm.	5.15	Heavy
110	33	No sulphur	7.45	Slight

NOTE:—*No oxygen was mixed with the hydrogen. No glass-wool filter was used.*

Good blanks could not be obtained using paraffin as a wall poison. The paraffin was tested and found to contain traces of sulphur. This confirmed the results obtained in blank runs. If the paraffin near the electrodes became warm a brownish yellow substance, which resembled vaseline, was formed.

The preceding data from a few of the many runs made in this series show that more sulphide was formed for those runs where the sulphur tube was in the line. The amount of sulphide formed in runs No. 107, 108 and 110 was approximately the same as for the blanks for the paraffin coated tube.

Experiments with Vacuum Discharge Tube Coated with Stearic Acid

The blanks with this tube for one-hour runs showed no visible trace of sulphide, but sulphide was formed when the activated gas passed over flowers of sulphur. In some cases when this was followed with a blank it was found that the tube had been contaminated by sulphur blown back.

To eliminate this trouble plastic sulphur was used in place of flowers of sulphur as testing agent; the plastic variety exposing much less surface to the activated gas. However, plastic sulphur is formed with an absorption of energy and might show more reactivity per unit of surface exposed.

Plastic sulphur was prepared by heating flowers of sulphur to 350° C. in a muffle furnace, and pouring the molten mass slowly into cold water. The rubber-like strings were pulled into small pieces and inserted in a tube coated with stearic acid. The water in which the plastic sulphur was made was tested for sulphides and no trace obtained. Blanks were run with hydrogen passing over the plastic sulphur without the discharge operating. No visible trace of sulphide was obtained in any of these blanks.

80 81 82 83 84 85 86

FIG. 2. *Results with some of the papers used in the test for sulphides in the preliminary work.—No. 80. Blank, oxygen mixed with hydrogen. No. 81. Water formed in tube, altering the discharge; sulphur became wet. No. 82. Blank, no oxygen added. No 83. Sulphur 2 cm. from discharge; oxygen mixed with hydrogen. No. 84. Same as No. 83, but with less oxygen. No. 85. Same as No. 83 and 84, but with less oxygen than No. 84. No. 86. Blank, with oxygen but no sulphur.*

144 145 146 147 148 149 150 151 152 153

FIG. 3. *A series of sulphide papers made during 0.5-hr. runs by the a.-c. corona discharge using plastic sulphur 4 cm. from the discharge. No. 144. Oxygen, 0.08%. No. 145. Blank, discharge on, no sulphur used. No. 146, 147, 148. Oxygen, 0.10%. No. 149. Oxygen, 0.12%. No. 150. Blank, same as No. 145. No. 151, Oxygen, 0.14%. No. 152. Oxygen, 0.10%. No. 153. Oxygen, 0.14%.*

The following data taken from part of the series show how effectively plastic sulphur eliminates the trouble experienced with flowers of sulphur. All subsequent data were obtained with fresh plastic sulphur as testing agent and a fresh lot was made for nearly every run. In a few cases one sample was used for two runs. No trouble with contaminated tubes was encountered in subsequent work.

The time for these runs was 15 min., except in No. 141 and 143, which were 30 and 45 min. respectively.

TABLE III

THE EFFECT OF COATING WALLS OF THE TUBE WITH STEARIC ACID ON THE FORMATION OF ACTIVE HYDROGEN USING PLASTIC SULPHUR AS TESTING AGENT

No.	Pressure in mm. Hg.	Distance of sulphur from discharge tubes	Velocity of hydrogen in litres per hr.	Sulphide
136	27	No sulphur	4.4	None
137	27	3 cm.	6.3	Heavy
138	24	No sulphur	4.3	None
139	26	3.4 cm.	5.1	Heavy
140	31	No sulphur	5.2	None
141	30	No sulphur	5.9	None
142	32	2 cm.	5.5	Heavy
143	32	No sulphur	5.0	None

Quantitative Estimation of Active Hydrogen

From the appearance of the sulphide formed on the test papers, it was considered that the quantity was large enough to show some reduction towards a dilute potassium permanganate solution. During a run, a known volume of permanganate solution, standardized with Kahlbaum's special sodium oxalate (99.9% pure), was used and the excess titrated with 0.01 N ferrous ammonium sulphate prepared from a previously analyzed salt. The ratio of the concentration of these two solutions was checked every few days.

In some of the reports concerning the properties of triatomic hydrogen, it is stated that this active gas will reduce potassium permanganate solution. To be consistent in this study, the use of blanks was continued. When it was found upon titration that some permanganate had been reduced during the blank runs, the presence of an impurity other than a sulphur bearing compound was suspected. The hydrogen was passed through a solution of potassium permanganate and two drying towers before it entered the line and purifying system. This made no difference in the amount of permanganate solution reduced. The gas was passed through the discharge but the blank did not change appreciably. In every blank run it was found that a small amount of the permanganate solution was reduced. If this solution were to be used to titrate the hydrogen sulphide formed by the active constituent, then blanks should be run under similar conditions to those maintained during runs with plastic sulphur in the line. Blanks were run for one hour, with velocities of hydrogen from 1.5 to 5 litres per hour, at pressures from 20 to 80 mm. Very little variation was found when comparing the amount of permanganate reduced

in one-hour runs. If the time was altered, the greatest variation was found.

Just and Kauko (11), and Wilke and Kuhn (28) have made investigations of the reduction of permanganate solution by hydrogen. The latter found that the time of contact of hydrogen with permanganate is one of the main factors influencing the amount of reduction. There was always much more hydrogen bubbling through the permanganate solution at the velocities and pressures used by the writers than was required for the reduction or reaction. This would indicate that for constant time all blanks would be equal. This was found to be the case throughout the series of blanks. They were run with the gas passing through the discharge under the same conditions as in regular runs except that there was no sulphur in the line. The blanks for one-hour runs for both corona and vacuum discharge were the same within experimental error.

Results

The curve in Fig. 4 was obtained from data secured from the operation of corona discharge on mixtures of hydrogen and oxygen. The activated gas was passed over plastic sulphur, 4 cm. from the discharge, using no glass-wool filter. No wall poison was used except water vapor formed in the discharge from the oxygen admitted, and the velocity of hydrogen was 12 litres per hour. Each point located is the average of from three to five runs. One drop of the permanganate solution was the widest variation from the average in the runs locating each point. The amount of reduced permanganate is plotted against the flow of oxygen. The flow of hydrogen was kept constant at 12 litres per hr. and the oxygen velocity varied. A definite optimum was found at about 0.1% of oxygen mixed with the hydrogen. This confirmed the qualitative evidence secured on the relation of added oxygen to the formation of sulphide as shown in Fig. 2.

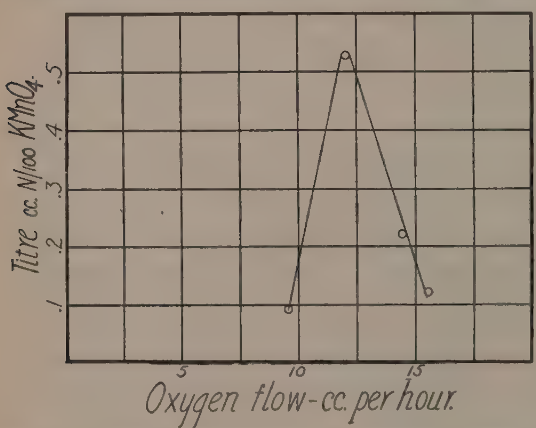

FIG. 4. *Relation between amount of potassium permanganate reduced and flow of oxygen, obtained from data secured from the operation of corona discharge on mixtures of hydrogen and oxygen.*

The curves in Fig. 5 and 6 were obtained from vacuum tube data, using the tube previously described. Purified hydrogen was used and walls of the discharge tube and those of the tube containing plastic sulphur were coated with stearic acid. The sulphur tube was placed 1.5 cm. from the discharge and no glass-wool filter was used. The discharge tube was immersed to a point above the electrode in an ice-water bath during the collection of data.

In Fig. 5, is plotted the amount of $N/100$ potassium permanganate solution reduced by hydrogen sulphide, against the velocity of hydrogen in litres per hour. The pressure in the tube was approximately 42 mm. The optimum velocity appears at close to 2.4 litres per hour.

In Fig. 6, is plotted the per cent activation against the pressure in millimetres over the range of pressures at which the tube was operated. The velocity was kept constant at 2.4 litres per hour. This is the optimum shown in Fig. 5.

Discussion

The evidence presented in the experimental work just described shows that hydrogen is activated in an alternating-current corona discharge at atmospheric pressure, if a small percentage of oxygen is previously mixed with it.

It also shows that hydrogen especially free from oxygen can be activated in a vacuum discharge at pressures from 20 to 80 mm. if the walls of the discharge tube have been poisoned with stearic acid.

It shows further that the fatigue effect manifested in this and some previous studies, using the above types of discharge, is influenced by the amount of oxygen present and the condition of the glass surface. This evidence is in harmony with the theory of R. W. Wood (29-32), who found that the presence of water vapor or oxygen favored the formation of the Balmer spectrum, and the exclusion of the secondary spectrum except near the electrodes. With dry hydrogen the secondary spectrum predominates. The catalytic effect of the glass walls in a discharge tube containing dry hydrogen caused the reversion of atoms to the molecular state. With moist hydrogen or with hydrogen containing a small concentration of oxygen, the walls were poisoned and the atoms were free to live their normal life period. Urey and Smallwood (22) consider that the fatigue effect found in all tubes was a factor influencing the uncertainty of their results. Wood (29-32) found that some metals which ceased to catalyze atomic hydrogen were restored to the initial state by exposing them to a discharge through air. It was found in this work that the most effective method for rejuvenating a spent tube is to fill it with air and operate the discharge for 15 min. The fatigue effect can be prevented by the previous addition of oxygen to the hydrogen or coating the glass surface with stearic acid. Anderegg and Herr (1) tried the addition of 1 part in 10,000 of oxygen but without effect. From the writers' experience this is too low a concentration. The sporadic tests for sulphide obtained by Urey and Smallwood (22) may be explained by assuming that the hydrogen contained a small concentration of oxygen. This may apply more particularly to their work with the corona tube. With this tube they used velocities from 12.8 to 84 litres per hour but they do not state the temperature to which they heated the platinized asbestos. Mitchell and Marshall (16) used a velocity of 2.1 litres per hour and heated the platinized asbestos to 300° C. The authors found that if the temperature of the platinized asbestos was raised to 550° C., the fatigue effect appeared in considerably less time. Then, associated with this change in fatigue effect, was the fact that less sulphide and finally perfect blanks were obtained. These

changes took place with the activated gas passing through sulphur. This indicated that the oxygen concentration might be a factor influencing the results of Urey and Smallwood. In their vacuum tube work a minimum velocity of 1.2 litres per hour was used. At this flow the platinized asbestos would remove the oxygen more efficiently. They obtained no sulphide under these conditions. This was the result found by Mitchell and Marshall and duplicated in the present work. The latter workers also found that a trace of oxygen would promote the activation of hydrogen and concluded that a small amount of the triatomic variety was formed.

In a discharge through dry hydrogen freed from oxygen the gas may be activated, but a test for it proved negative due to the catalytic effect of the glass walls. The experiments with dry hydrogen in a vacuum tube, the walls of which were poisoned with stearic acid, show that hydrogen can be activated if the metastable constituents are protected from the glass surface.

To duplicate the work of Wendt and Landauer (27), it is necessary to use hydrogen containing a small percentage of oxygen. The corona discharge through hydrogen containing 0.1% of oxygen forms the active gas. The concentration, using a velocity of 12 litres per hour and plastic sulphur 4 cm. from the discharge, was of the order of 0.001%. It would be expected that, with the sulphur tube closer to the discharge, the concentration of active gas would rise. In these experiments the maximum with the vacuum tube discharge, using a hydrogen velocity of 2.2 litres per hour and plastic sulphur tube 1.5 cm. from discharge, was 0.025%. This checks very well with the work of the above authors.

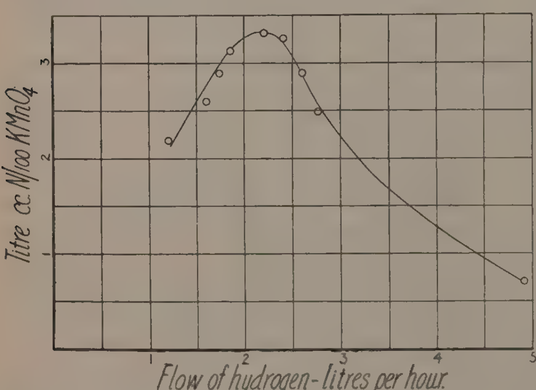

FIG. 5. *Relation between amount of potassium permanganate reduced and flow of hydrogen. Plotted from data secured with the walls of the vacuum tube coated with stearic acid.*

If we consider the relation of oxygen concentration to the amount of active constituent from the data plotted in Fig. 4, it is found that hydrogen sulphide is formed at a concentration of oxygen sufficient to cover the glass surface of the corona tube with a layer one molecule thick. Part of this molecular layer may be water but Wood (29-32) has assumed that oxygen plays the part of protector; the optimum effect appears near 0.1% of oxygen. The volume of the tube was 80 cc. and the diameter 1.25 cm. Debeye's value for the molecular diameter was used. Hydrogen, especially free from oxygen and dried by phosphorus pentoxide, contains less water vapor than the concentration necessary to cover the walls of the corona tube. It was under these conditions that all the tests were negative with unpoisoned walls.

The curve in Fig. 5 was plotted from the data secured by using the vacuum tube with walls coated with stearic acid. The hydrogen used was especially free from oxygen. The curve shows the formation of an active unstable constituent in the discharge tube. Assuming this to be triatomic hydrogen, it is found that, at the lower velocities, some of active gas decays before it reaches the sulphur. Then as the velocity is increased, the time of transfer from the discharge to the sulphur decreases, thereby increasing the amount of triatomic gas reaching the sulphur and undergoing reaction. The optimum was reached at a velocity of 2.4 litres per hour. At higher velocities the time the gas spends in the discharge appears to be insufficient to form this active constituent in optimum amount. This is what one would expect in the formation of a complex group such as triatomic hydrogen. Conditions (23) which favor the formation of H_3^+ also favor the formation of Fulcher bands. Some of the Fulcher bands are greatly intensified when the pressure in the discharge tube is raised to 50 mm. (15). Monk and Elo (17) attribute some of the bands in the secondary spectrum of hydrogen to H_3.

In Fig. 6, we have further verification of the formation of an activated unstable constituent. The data are from the same tube that was used to secure the results shown in Fig. 5. The velocity of hydrogen was 2.4 litres, the optimum of the previous curve. The pressure was varied from 30 to 80 mm. by regulating the rate of pumping. Under these conditions the time the gas spent in the discharge increased as the pressure increased. But the rate at which gas passed from the discharge to the sulphur tube decreased. Because of this reduced rate, increasing quantities of the active gas would become deactivated before reaching the sulphur. Also, at increased pressures collisions would be much more frequent, resulting in a further decrease in the percentage of active constituent found. The curve shows this condition. The rate of decrease of per cent active constituent with the pressure is greater at pressures above 50 mm. than it is from 30 to 50 mm. The per cent active gas multiplied by the pressure is approximately a constant up to 45 mm. but this product decreases very rapidly as the pressure increases. Of course, above 45 mm., the optimum values for each pressure have not been plotted. The variation in the slope

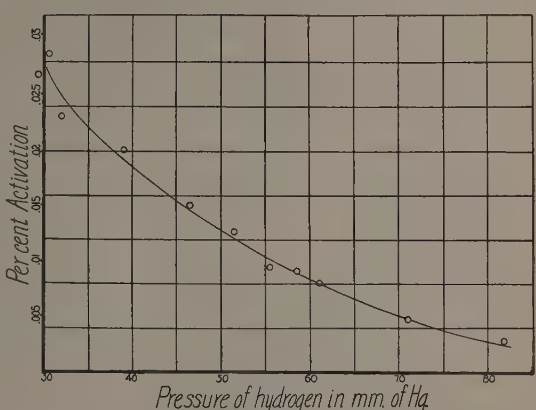

FIG. 6. *Relation between per cent activation and pressure in mm. over the range of pressures at which the tube was operated. The data are from the same tube used to obtain results shown in Fig. 5.*

of the curve in Fig. 7 would seem to indicate that optimum values multiplied by the per cent activation would give a constant.

Elliott's (8) work presents more of a contrast than a comparison with the results of the authors. He finds that the yield of active hydrogen increases with a decrease in velocity. The results of this investigation are just the reverse below the optimum velocity. Elliott finds no optimum where the yield and velocity are at a maximum. In explaining the increase in yield despite the low velocity of the short-lived gas, he considers that the time the gas spends in the discharge more than compensates for the decay of the active constituent in traversing the distance to the sulphur. With each decrease in velocity the gas spends a longer time reaching the sulphur. It would seem then that there should be an increase in the amount decaying and a decrease in the amount formed as observed by the test.

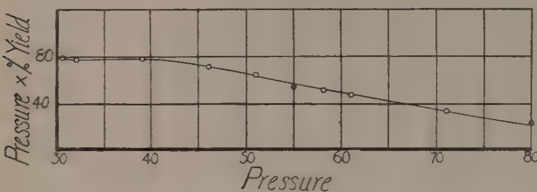

FIG. 7. *Relation between pressure multiplied by percentage yield of active constituent and pressure.*

If we consider the lowest velocity in Table I, page 67 of Elliott's paper, in relation to the time to traverse the smallest volume between the sulphur and the discharge given in Table V, page 73, namely 0.5 cc., we find that at the pressure inside the discharge the gas would pass from discharge to the sulphur in 0.9 seconds. Now, in Table V, he shows that practically all the active gas decomposes in 0.2 seconds. Therefore, it seems to the writers that the yield of active hydrogen should decrease with the decrease in velocity as they have found it to do.

Elliott also assumed that the percentage variation of the yield of active hydrogen is independent of the pressure. The authors' results show that there is a considerable variation of the percentage yield with the pressure.

Since glowing nitrogen has been found to contain a chemically active constituent other than the one emitting the glow, and hydrogen, subjected to the discharge, contains more than one type of positive ion, a disagreement in results does not obviate the possibility that more than one type of active hydrogen may be involved in the discrepancies. It is trusted that future investigation will elucidate this point.

Summary

1. It has been shown that hydrogen especially free from oxygen may be activated in a vacuum tube discharge if the walls are poisoned with stearic acid.

2. Hydrogen containing 0.1% of oxygen is activated by both the corona discharge at atmospheric pressure and the vacuum tube discharge at pressures from 20 to 80 mm.

3. The fatigue effect appearing in the discharge tubes during this study of the activation of hydrogen has been shown to be related to the oxygen content of the gas and to the surface condition of the tube.

4. The quantitative and optimum relations between yield and velocity at constant pressure, and the variation in per cent of activation with the pressure, have been studied.

5. An explanation has been proposed for the discrepancies existing regarding the work of some previous investigators.

6. Plastic sulphur has been found to be a better testing agent for active hydrogen than flowers of sulphur.

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STUDIES OF POLYMERS AND POLYMERIZATION

II. THE PYROLYSIS OF POLYINDENES AND THE POLYMERIZATION OF DIINDENE[1]

By George Stafford Whitby[2] and Morris Katz[3]

Abstract

On heating samples of polyindene to temperatures below those necessary to effect pyrolytic distillation, they suffer cracking, as evidenced by a fall in molecular weight. Such cracking takes place the more readily, the higher the molecular weight of the sample taken. On subjecting polyindene to slow pyrolytic distillation at 2 mm. up to bath temperatures of 400° C., there is obtained a distillate containing indene, diindene and triindene. The residue consists of low polymers. Under these conditions cracking is more profound, *i.e.*, the amount of distillate is larger, in the case of a polyindene of high molecular weight than in the case of one of relatively low molecular weight. On pyrolytic distillation at ordinary pressure, diindene yielded 74% of indene. Diindene can be polymerized by sulphuric acid, by antimony pentachloride and by heat. A mixture of mono- and di-indene yields triindene, when heated. It is concluded that the results accord better with the authors' formula for the polyindenes rather than with Staudinger's ring formula. In the author's view the polyindenes are open-chain compounds which owe their formation to a series of addition reactions involving the wandering of hydrogen, and, conversely, that their pyrolysis is a cracking reaction also involving wandering of hydrogen.

In Part I of this series (11) it was shown that there exists an unbroken series of polyindenes representing indene in various stages of polymerization, and that polyindenes, whether prepared by heating indene or by treating it with catalysts such as stannic chloride or antimony pentachloride, are highly heterogeneous. Heterogenity has also been demonstrated by Staudinger (2, 3) in the case of polyindene prepared by means of stannic chloride. It was also shown in Part I that samples of polyindene ranging in molecular weight from 414 to 3320 all absorbed bromine to the extent of two atoms per mole, and there was suggested a scheme for the mechanism of the polymerization of indene and structural formulas for the polyindenes.

It seemed probable that a study of the pyrolytic decomposition of the polyindenes would offer further evidence concerning their constitution. In the case of polymers, such as caoutchouc, derived from conjugated dienes, pyrolysis throws little light on the structure, because, as one of the authors has pointed out previously (10), cyclisation takes place, owing to the occurrence of a double bond at every fourth carbon in the chain. Such a complication is absent in the case of polymers of indene and styrene.

Experiments on the pyrolytic decomposition of polyindenes at ordinary pressure have been reported by Kraemer and Spilker (1), by Weger (7) and by Stobbe and Färber (5), and at reduced pressure by Stobbe and Färber and by Staudinger, Ashdown, Bruson and Wehrli (3). For the purpose of studying the

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Contribution from the Department of Chemistry, McGill University, Montreal, Canada. This work was carried out in 1928.

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pyrolysis of polyindenes more closely, it seemed desirable to employ low pressures, in order to isolate the initial scission products as completely as possible. Although diindene was found to be distillable without substantial decomposition under pressures of 25 mm. and less by Stobbe and Färber (5) and in the present experiments, it breaks down when heated at ordinary pressure, yielding 74% of indene (see Experimental Part). Distillation under a pressure of 20 mm. and at a bath temperature of 325-350° C. of a polyindene having a molecular weight of the order of 2000 was found by Stobbe and Färber to yield only 25% of distillate. In the present studies a pressure of only 1-2 mm. was employed. Further, the pyrolysis was carried out slowly and with care to prevent overheating, so as to avoid as far as possible the formation of truxene (tribenzylene benzene). The desirability of this precaution is shown by the fact that heating polyindene with a free flame is recommended by Stobbe and Zschoch (6) as a good method of preparing truxene.

By slow and careful heating under a pressure of 2 mm., it was found possible to obtain from suitable preparations of polyindene a distillate representing more than 80% of the material taken. The distillate consisted of indene, diindene, triindene and a small amount of even higher polymers. The residue, which could not be distilled over without raising the temperature to a point at which charring occurred, contained polyindenes higher in molecular weight than the trimer but not so high as the original material. The amount of truxene formed was relatively small.

The results in Table I were obtained with a sample of polyindene prepared by treating indene with stannic chloride and having a molecular weight of 2065.

TABLE I

Distillation of polyindene of mean molecular weight 2065 under pressure of 2 mm.

	Distillate (on redistillation), %	Residue, %	Total, %
Indene	30.5		30.5
Diindene	20.6		20.6
Triindene	15.0	2.1	17.1
Low polymers (fractions of m.w., 474-891)		7.9	7.9
Truxene, etc.	0.6	10.6	11.2
Loss			12.7
			100.0

In view of the fact that polyindenes as prepared from indene by catalysts or by heat are mixtures of material in various stages of polymerization, and the consequent uncertainty as to whether the low polymers isolated after heating the sample employed in the experiment just mentioned were present originally or were produced during the pyrolysis, a similar sample of polyindene was freed from material in a low state of polymerization, rendered as nearly homogeneous as possible, and subjected to pyrolysis. The material, which had a molecular weight of 2631, yielded monomer, dimer, trimer and also a series of low polymers ranging in molecular weight from 400 to 655. This result made it clear

that, not only the distillable, but also the residual, undistillable, low polymers, are derivable by the cracking of a high polymer.

In order to ascertain the influence of the degree of polymerization on the ease and course of depolymerization, a fraction of low molecular weight (608) was prepared in as homogeneous a condition as possible, and its behavior on pyrolysis compared with that of the high molecular fraction just mentioned. It was found to undergo depolymerization less completely and less profoundly than the fraction of high molecular weight. Whereas the distillate obtained from the latter represented 82% of the material, that from the former represented only 43.5%, and, further, it was necessary in the case of the low fraction to raise the bath temperature to 390° C. or 30° C. higher than the maximum bath temperature reached in the distillation of the high fraction. The results are summarized in Table II.

TABLE II

Pyrolysis under 2 mm. pressure of two "homogeneous" samples of polyindene of molecular weights 2631 and 608 respectively

	Yield, %					
	High polyindene			Low polyindene		
	Distillate (on redistillation)	Residue	Total	Distillate (on redistillation)	Residue	Total
Indene	39.5		39.5	13.2		13.2
Diindene	16.1		16.1	10.8		10.8
Triindene	22.3		22.3	12.0		12.0
Low polymers	1.6*	8.7†	10.3	5.1§	39.8‡	44.9
Truxene, etc.	1.1	5.7	6.8	1.7	14.9	16.6
Loss			5.0			2.5
			100.0			100.0

* *Mol. wt., 405.* †*Mol. wt., of three fractions into which this was split: 657, 531, 393.* ‡*Mol. wt., 630.* §*Mol. wt., 500.*

A sample of thermo-polymerized indene, when pyrolyzed under 2 mm. pressure, behaved similarly to polyindene prepared by means of stannic chloride, yielding a distillate consisting of indene, diindene and triindene and leaving an undistillable residue of low polymers. Although the sample had a mean molecular weight (600) similar to that (608) of the low "homogeneous" polymer just mentioned, the products of pyrolysis appeared in different proportions. A smaller proportion remained as undistillable low polymers and a larger proportion came over. This was doubtless due to the fact that, although the mean molecular weight of the two materials was substantially the same, the heat polymer was markedly heterogeneous and contained a considerable proportion of polyindene fractions with molecular weights of the order of 1000 (Cf. (11) p. 1168, Table III). The results of the pyrolysis of the thermo-polymer are summarized in the following table.

In order to secure data for a more complete picture of the course of the pyrolysis of polyindene, a sample of polyindene of high molecular weight (2631)

TABLE III

PYROLYSIS OF A SAMPLE OF THERMO-POLYMERIZED INDENE OF MOLECULAR WEIGHT 600.

	Distillate (on redistillation), %	Residue, %	Total, %
Indene	21.1		21.1
Diindene	19.6		19.6
Triindene	24.3		24.3
Low polymers		23.1*	23.1
Truxene	1.6	6.1	7.7
Loss			4.2
			100.0

**Separated into two fractions: (a) 19.9%, molecular weight, 567;*
(b) 3.2%, molecular weight, 451.

and one of low molecular weight (about 600) were heated for various periods at temperatures lower than those applied in the distillation experiments already considered. At 177° and at 197° C. the high molecular material suffered a large reduction in molecular weight, whereas the molecular weight of the low polymer was unchanged. At 214° C., while the high molecular material suffered in 15 days a reduction in molecular weight from 2631 to 870, the low polymer was affected only slightly, the molecular weight changing from 608 to 562. The results are collected in Table IV.

TABLE IV

EFFECT OF HEAT ON THE MOLECULAR WEIGHT OF SAMPLES OF POLYINDENE OF HIGH AND OF LOW INITIAL MOLECULAR WEIGHT

Temperature, ° C.	Time, days	High polymer		Low polymer	
		Mol. wt. before	Mol. wt. after	Mol. wt. before	Mol. wt. after
177	15	2631	1180	639	642
177	28	2631	903	608	623
197	5½	2631	1558	608	604
197	15	2631	1017	608	588
214	5½	2631	1360	608	583
214	15	2631	870	608	562

It is clear that polyindene of higher molecular weight undergoes scission to smaller molecules under the conditions applied more readily than polyindene of lower molecular weight. This is in accord with the principle which appears to hold for cracking in general, that, for compounds of the same chemical type, *e.g.*, the straight-chain paraffins, cracking occurs more readily the larger the carbon chain*.

Observations by Staudinger, Johner and Wiedersheim (4) on the effect of heat on samples of polyindene are also in accord with this principle. These authors measured the viscosity of solutions of three samples of polyindene before and after heating the samples at 270-280° C. for 10 hr. and found that,

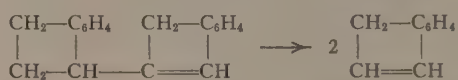
**For data illustrative of this principle, see The Pyrolysis of Carbon Compounds by C. D. Hurd, New York, 1929.*

whereas a sample of molecular weight 1800 was almost unaffected and indeed showed a slight increase, a sample of molecular weight 3000 showed a very marked reduction in the viscosity of its solutions (indicating a reduction in molecular weight) and one of molecular weight 6000 a still greater reduction. Stobbe and Färber (5) had previously stated that a polyindene with a molecular weight of the order of 2000 showed a slight increase in molecular weight as a result of long heating at 200° C. but gave no details of their experiments in this connection.

It would appear that at the temperature applied in the distillation experiments on polyindene previously referred to, only polyindenes with a molecular weight corresponding to or lower than $(C_9H_8)_6$ are capable of surviving without cracking over the period of distillation (about eight hours).

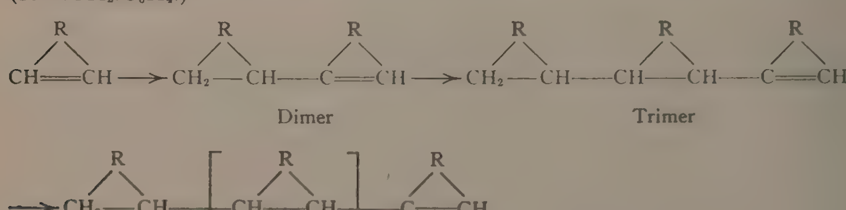
The pyrolysis of diindene was examined by heating it at ordinary pressure. It was found to crack very largely to indene, the amount of the latter which distilled over being 74% of the weight of the diindene. Further, depolymerization did not begin until the bath temperature reached 340° C. Hence it would appear that the indene obtained by the pyrolysis of samples of polyindene (supra) did not come from the cracking of indene, since the bath temperature at which it was obtained (165 to 230° C. in different cases) was much lower. It would appear that the indene obtained by the pyrolysis of polyindenes split off directly from molecules much larger than the molecule of the dimer.

The pyrolysis of diindene in the main follows the reaction—



It is thus a typical cracking reaction, involving the wandering of hydrogen and is the strict converse of the polymerization of indene to diindene. In the authors' view the conversion of indene to its high polymers on the one hand and the cracking of polyindenes to indene, diindene, triindene, etc., on the other hand similarly involve the wandering of hydrogen.

The formation of the polyindenes is considered as involving the stepwise addition of indene molecules with the wandering of hydrogen, as follows (11):—
$(R = .CH_2.C_6H_4.)$

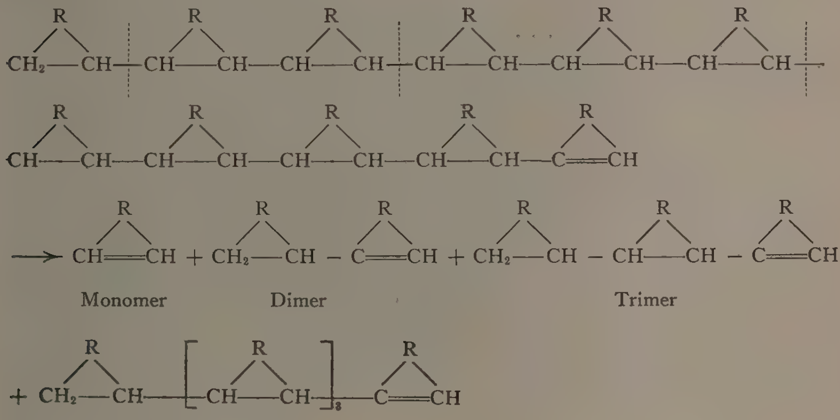


Dimer Trimer

Polymer

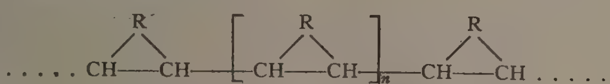
A consideration of the results obtained in the present investigation on the thermal decomposition of polyindenes makes it probable that scission does not

occur in a strictly stepwise manner, as, *e.g.*, $(C_9H_8)_{16} \longrightarrow 2(C_9H_8)_8 \longrightarrow 4(C_9H_8)_4 \longrightarrow 8(C_9H_8)_2 \longrightarrow 16\ C_9H_8$. It seems probable that the cracking of high polyindenes involves the scission at an early stage of monomeric indene from the end of the long chain molecules; the scission, as the temperature is raised, of diindene, and, later, scission of the chain at several points simultaneously, as, *e.g.*,



Pentamer

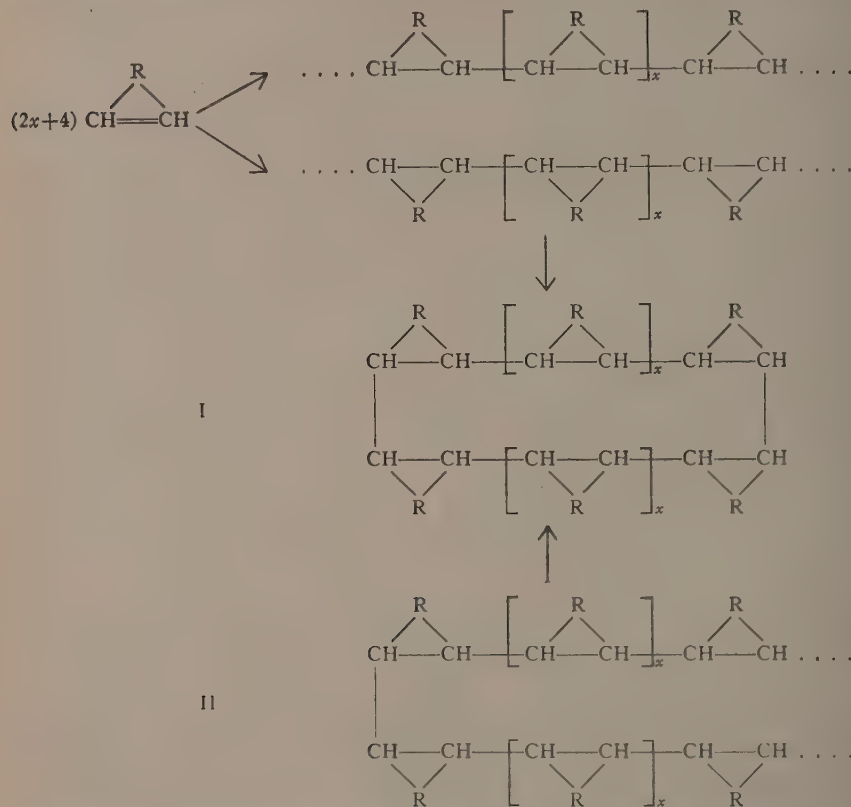
An open-chain formula with terminal free valencies was formerly favored by Staudinger for polyindene (2)—



Objections to this formula, based not only on general considerations, but also on the finding that polyindene fractions, irrespective of their molecular weight, contain one double bond and absorb two atoms of bromine per mole, were formulated in the first paper of this series. More recently (3) Staudinger has expressed himself as favoring ring formulas for the polyindenes. He supposes that long chains of indene first form, and that two of such chains unite through the free end valencies which are assumed to be present, giving rise to rings (I) if they unite at both ends or to structures such as II if they unite at one end only. Staudinger's scheme is shown on page 350.

It is difficult to see how a formula such as II differs from Staudinger's original formula, and the same objections apply to it as to the latter. The formulation of polyindenes as rings is, like their formulation on the lines previously followed by Staudinger, out of accord with that fact that the polyindenes contain one double bond per molecule, as shown by measurements of their bromine absorption. This fact, previously established for samples obtained by heating indene and ranging in mean molecular weight from 414

to 948 and for samples obtained by the action of stannic chloride on indene and ranging in molecular weight from 952 to 3320, has been confirmed in the present experiments for diindene, triindene and low polymers of molecular weight 474 to 892 obtained by cracking polyindene with an original molecular weight above 2000.

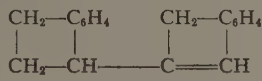


The very existence of an unbroken series of polymers from $(C_9H_8)_2$ to $(C_9H_8)_{ca30}$, generally similar in properties and behaving similarly on pyrolysis, accords much better with an open-chain formula such as the authors' than with a ring formula. The formulation of the polyindenes as rings involves the idea that rings of 4, 6, 8, 10 . . . 60 carbon atoms present no striking differences in the ease with which on the one hand they form and on the other hand break down.

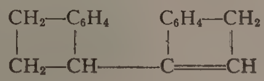
It is difficult to envisage the process of cracking on the basis of the ring formula. It would appear necessary to assume that, when a high molecular polyindene gradually falls in molecular weight on being heated, the ring opens; the open chain then cracks, and the fragments form smaller rings. If indeed the high polyindenes consisted of large rings, of, say, 60 carbon atoms, it would

seem more reasonable to assume that, following ring fissure, the valencies of the chain would rearrange themselves and a chain structure, with a double bond, similar to that represented by the authors' formula, would arise.

The two lowest polymers, diindene and triindene, clearly fall in line in the series of polyindenes, as is shown by their melting points and other properties, including behavior on pyrolysis. Yet neither is a cyclic compound. The structure of diindene has been shown by Stobbe and Färber (5) to be either III or IV—



III



IV

The former structure is to be preferred to the latter, since diindene is on the direct line to the higher polymers in the formation of which it is most reasonable to assume the addition of indene molecules to each other occurs in a regular way, *i.e.* in a way such that each successive molecule adds on in the same sense as the previous one.

It may be noted that the fact that diindene and triindene—definite, crystalline chemical individuals—were obtained as scission products of polyindene shows that in the polymeric forms obtainable by heating indene or treating it with catalysts indene units are combined not merely by "association forces" but by primary valencies.

Additional evidence concerning the mechanism of the polymerization of indene is afforded by the observation that diindene is itself polymerizable and that indene and diindene when heated together are capable of undergoing addition to form triindene. Diindene was found to be convertible to polymers by the same agencies as those which effect the polymerization of indene, although with less readiness. Antimony pentachloride gave a polymer with a molecular weight of 1233; sulphuric acid, a polymeric product corresponding approximately to a hexamer.

TABLE V

THERMO-POLYMERIZATION OF DIINDENE

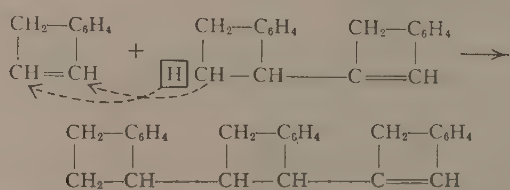
Period of heating, days	Polymerization, %	M.p. of polymer, ° C.	Mol. wt. of polymer
At 214° C.			
8	24.6	77–80	373
15	58.5		
21	81.4	119–123	562
At 238° C.			
3½	18.3	72–75	352
8	39.0	83–87	407
15	74.8	95–97	447
21	84.5	102–106	492

On heating diindene at 179° C. for 11 days, it remained unchanged. (Under these conditions indene was previously (11) found to change to the extent of 65.5% to a polymer of molecular weight 566.) On heating diindene at 214° and at 238° C., however, it underwent polymerization. The results are shown in summary in Table V.

A comparison of these results with those previously obtained for the thermo-polymerization of indene (11) shows that diindene requires a higher temperature for its polymerization than does indene. For example, the rate of polymerization of diindene at 238° C. is similar to that of indene at 178° C.

As was found in the thermo-polymerization of indene, so in that of diindene, (a) the molecular weight of the product rises as the percentage which has polymerized increases, (b) the molecular weight of the polymer produced when the conversion approaches completion is lower, the higher the temperature applied.

A mixture of equal parts of indene and diindene heated for 15 days at 177° C. yielded 33% of triindene and a similar mixture heated for 5½ days at 197° C., 46.6% of triindene. The balance of the material was isolated in each case as unchanged indene and diindene. The amount of diindene which had disappeared was in both cases equivalent to the triindene obtained. The temperatures used are below those at which diindene alone undergoes any appreciable amount of polymerization. These experiments would appear to prove that one molecule of diindene can add on to a molecule of indene, or *vice versa*, to yield triindene.



This reaction represents one of the stages in the stepwise conversion of indene to high polymers according to the scheme previously outlined, which involves a succession of addition reactions, hydrogen from one molecule going to one side and the rest of the molecule to the other side of a double bond present in another molecule. This scheme has been criticized by Staudinger (3) on the ground that the double bonds in the intermediate members of the series —diindene, triindene, etc.—are insufficiently reactive to enable them to add on indene molecules under the conditions in which polymerization occurs. The present investigation, in which it has been shown not only that diindene is itself polymerizable, but also that indene and diindene can unite to form triindene, does not support this contention. Further, the succession of steps leading to the formation of polyindenes are not necessarily to be conceived as involving the addition of indene molecules to the intermediate stages; more probably they involve to a large extent the converse of this, namely the addition of the intermediate stages to indene in a manner similar to that indicated in the last equation.

Experimental

Pyrolytic Distillation of Polyindenes

Heterogeneous Polymeric Product

Experiment I. In this preliminary experiment 26.4 gm. of polyindene prepared as previously described (11) by treating indene with stannic chloride in chloroform solution and purifying the polymer by three precipitations with alcohol from benzene solution, was heated gradually, by means of a bath of Wood's metal, in a 50-cc. Claisen distilling flask to which was attached a condenser, the inner tube of which was 40 cm. by 6 mm. A pressure of 2 mm. was maintained. The molecular weight of the polyindene taken was 2200 (0.7100 gm. in 17.58 gm. of benzene gave a depression of 0.094° C.) and its melting point 225-9° C.

The temperatures over which distillation took place were as follows:— bath temperature, 262° to 365° C.; temperature of flask contents, 230 to 360° C.; temperature of distillate, 110 to 180° C. When the temperature of the contents of the flask reached 360° C. no further distillate could be obtained without charring. The weight of the distillate was 20.10 gm.

The portion of the residue in the distilling flask which was soluble in hot benzene was precipitated twice from benzene solution by means of absolute alcohol. It was thus obtained as a yellow powder weighing 1.6 gm. and melting at 343-347° C. It was not soluble enough in benzene, camphor, etc. for a molecular weight determination and was taken to consist mainly of truxene.

The distillate was separated into the following fractions by distillation at 2 mm. *1.* 6.75 gm. obtained at a bath temperature of 40-45° C. This boiled at 35-37° at 2 mm. and 181-2° C. at atmospheric pressure. These figures agree with those obtained for pure indene. *2.* After the last trace of monomer had come off, the water bath was replaced by a metal bath and the temperature of the bath raised to 195° C.; 4.80 gm. of a viscous golden yellow oil distilled over at 160-170° C. It crystallized on standing. Mol. wt. 223, 225 (calculated for diindene, 232). *3.* 3.6 gm. of a viscous red oil which resinified on cooling came over at 185-200° with a bath temperature of 250-270° C. Mol. wt., 331, 347 (calcd. for triindene, 348). *4.* The residue in the distilling flask was treated with ether. Addition of absolute alcohol to the ethereal solution precipitated 2.7 gm. of a polyindene as a yellow powder melting at 107-9° C. Mol. wt., 534, 504. The ether-insoluble portion of the residue appeared to be truxene; it weighed 2.5 gm. and melted above 340° C.

Experiment II. The results of this experiment are summarized in Table I. Another sample of polyindene (68.2 gm.) prepared in a similar way to the foregoing was distilled from a 250-cc. Claisen flask under 2 mm. Mol. wt. 2065. The pyrolytic decomposition occupied eight hours. The amount of distillate secured was 48.6 gm., the temperatures prevailing during the distillation being as follows:— temperature of bath, 251 to 360° C.; temperature of contents, 220 to 348° C.; temperature of distillate as shown by a thermometer in the side arm, 81 to 185° C.

Residue. The residue in the flask, about 19 gm., was heated with 250 cc. of benzene; 7.2 gm. was insoluble. Of this, 2.1 gm. was soluble in chloroform and was precipitated therefrom by alcohol. It consisted of a yellow powder, melting at 365-370° C. and insufficiently soluble in benzene and other solvents for molecular-weight determinations. The portion insoluble in both benzene and chloroform was taken to be mainly truxene. On treating the benzene solution of the residue with absolute alcohol, there was obtained 1.9 gm. as a precipitate, which, after being purified by a further precipitation consisted of a yellow powder. M.p., 178-182° C.; mol. wt., 865, 918. C, 92.86; H, 6.53% (calcd. for $(C_9H_8)_n$: 93.10, 6.90%). The melting point accords with the melting point of fractions of polyindene of similar molecular weight described previously (11).

On concentrating the mother liquor from which the above material was obtained and adding alcohol, a second fraction of polyindene was obtained weighing 3.5 gm. after purification and melting at 120-123° C. Mol. wt. 485, 463. C, 92.70; H, 6.57%.

Further concentration of the mother liquor to 50 cc. and the addition of absolute alcohol gave 1.4 gm. of a fraction corresponding to triindene. M.p. 65-68° C., mol. wt. 368, 357. The mother liquor still contained 1.0 gm. of material in solution, but this was discarded as too impure for observation.

Distillate. When distilled at 2 mm. this gave the following fractions. *1.* 20.8 gm. indene boiling at 35-38° C./2 mm. Mol. wt., 115, 113. C, 92.8, 92.9; H, 7.1, 7.05% (calcd. for C_9H_8: 93.10, 6.90%). *2.* 14.1 gm. distilling between 135 and 140° C. under 1-2 mm. at a bath temperature of 155-160° C. Mol. wt. 223, 221. C, 92.65; H, 7.01% (calcd. for $(C_9H_8)_2$: Mol. wt., 232; C,93.10; H, 6.90%). *3.* The red brown, extremely viscous residue in the distillation flask (11.3 gm.) was taken up in 100 cc. of ether. The ether-insoluble portion (0.43 gm.) was identified as truxene (8); m.p., about 360° C. C, 94.3; H, 5.53% (calcd. for $C_{27}H_{18}$: 94.74, 5.26%). Alcohol (250 cc., 95%) was added to the ethereal solution and the yellow, very viscous oil which separated was isolated. On drying, it changed to a yellow, sticky resin similar to the low heat polymers of indene. It was apparently impure triindene. Weight, 10.2 gm. Mol. wt., 278, 282. C, 92.5; H, 7.04%.

Homogeneous Polyindenes of High Molecular Weight

A fraction of high molecular weight, substantially free from material in a low state of polymerization, was prepared as follows. To a mixture of 500 cc. of indene and 500 cc. of chloroform contained in a flask attached to a reflux condenser and cooled by a bath at 10° C. was added slowly with shaking 50 cc. of a 20% solution of stannic chloride in chloroform. The liquid turned deep red and enough heat was evolved to cause the chloroform to boil. At the end of two hours, 1500 cc. of absolute alcohol was added to the syrupy solution of polyindene, the precipitate was collected, dried, dissolved in benzene and reprecipitated with alcohol. Reprecipitation was carried out three times. The product was 453 gm. of a white, amorphous powder, free from any trace of catalyst; m.p. 221-226° C.; mol. wt. 1873, 1941.

The product (450 gm.) was dissolved in 1200 cc. of chloroform and 200 cc. of alcohol added dropwise with mechanical stirring. The swollen, gelatinous polyindene which separated was isolated and kneaded and washed with alcohol until free from imbibed solvent. This fraction consisted of 399.1 gm. of a white powder melting at 227-233° C. Addition of one litre of alcohol to the mother liquor gave a second fraction weighing 40.4 gm. and melting at 202-209° C. Concentration of the mother liquor to 200 cc. gave a third fraction weighing 9.1 gm. and melting at 156-161° C. The first fraction (399.1 gm.) was then subjected to nine more fractionations* on similar lines and yielded 216 gm. of a fraction melting at 245-247° C. which was almost entirely free from lower polymers as shown by the following facts. Mol. wt. 2570, 2704, 2620; av. 2631. After this first fraction had been precipitated the addition of 800 cc. of alcohol to the filtrate gave only a slight turbidity, and on then evaporating the liquid to 100 cc. only 0.5 gm. of a polymer melting at 189-193° C. was obtained. Further, when the 216 gm. was separated into two fractions, the latter were identical in melting point.

Seventy-five grams was distilled at 2 mm. Distillation commenced at a bath temperature of 260°, the distillate boiling at 79° C. After one hour, distillation ceased and the bath temperature was increased to 300° C. Active distillation recommenced, a reddish oil boiling at 105-132° C. coming over and the temperature of the flask contents being 286° C. As the temperature of the bath was gradually raised to 360° C., that of the flask contents rose to 357° C. and of the distillate to 186° C. Most of the distillate came over between 105 and 155° C. After eight hours, distillation was stopped, as the contents of the flask began to show signs of decomposition, gases being produced which could not be condensed.

Residue. This weighed 13.4 gm. On treatment with 250 cc. of hot benzene, 4.3 gm., consisting mainly of truxene and decomposition products, remained undissolved. On cooling, the benzene solution deposited 2.2 gm. of material, which, unlike polyindene, was practically insoluble in cold benzene, and probably consisted of truxene. Three fractions of polyindene were obtained from the benzene solution as follows. (a) 2.6 gm. by concentration to 125 cc. and addition of 200 cc. of alcohol. After purification by two more precipitations from benzene solution, it melted at 140-142° C. Mol. wt., 661, 652. C, 92.9; H, 7.02% (calcd. for $(C_9H_8)_n$: 93.1, 6.9%). (b) 2.8 gm. by concentration of the mother liquor from the above fraction to 100 cc.; m.p., 111-114°C. Mol. wt., 538, 525. (c) 1.1 gm. by further concentration to 25 cc.; m.p., 80-83° C. Mol. wt., 393.

Distillate. This weighed 61.6 gm. By distillation at 2 mm. it was separated into four fractions as follows. *1.* 29.6 gm. of indene boiling at 35-40°C./2 mm. and at 181-182° C. when redistilled at atmospheric pressure. *2.* 12.1 gm. of a golden yellow oil which boiled at 150-163° C./2 mm. and after being twice crystallized from hot acetic acid was obtained as colorless crystals, melting at

**The following data referring to some of the lower melting fractions separated may be recorded. Fractions melting at 129-131°, 192-195° and 210-214° C. had molecular weights of 639, 1445, 1699 respectively.*

56-57° C., and identical with diindene. *3.* 16.7 gm. boiling at 222-230° C., which came over as a viscous red oil and thickened to a sticky resin on cooling. It was purified by dissolving in 75 cc. of benzene and throwing it out with alcohol. Mol. wt., 324. On crystallizing a portion from hot absolute alcohol (solubility: 1.2 gm. per 100 cc.), it was obtained as a fine yellow powder melting at 69-71° C. Mol. wt., 356. C, 93.05; H, 6.79 (calcd. for $(C_9H_8)_3$: 348, 93.10, 6.90). *4.* On treating the residue in the distilling flask with 50 cc. of hot benzene there was left 0.8 gm. of material with a melting point (350-360° C.) corresponding to that of truxene. By adding 125 cc. of absolute alcohol to the benzene solution, 1.2 gm. of material presumed to be polyindene was obtained. M.p., 86-89° C. Mol. wt., 400, 411.

Homogeneous Polymer of Low Molecular Weight.

The material used in this experiment was prepared from the lower melting fractions separated in the course of the preparation of the high polymer used in the preceding experiment. Eleven such fractions ranging in melting point from 137° to 177° C. were combined. The combined material (60.5 gm.) was subjected to two reprecipitations by alcohol from chloroform solution and gave 51.5 gm. of polyindene melting at 145-148° C. Mol. wt., 596, 619 (av., 608).

Thirty-five grams was subjected to pyrolytic distillation at 2 mm. over a period of eight hours. The temperatures over which distillation occurred were as follows:— Bath, 171 to over 400°; flask contents, 148 to 390°; distillate 92 to 260° C. The distillate amounted to 15.2 gm. and the residue in the flask to 19.8 gm.

Residue. The residue was treated with 200 cc. of chloroform and 5.2 gm. of insoluble material separated. Polyindene was precipitated from the solution by the addition, with stirring, of 650 cc. of alcohol. On concentrating the mother liquor to 50 cc., a further small quantity of polymer separated. It was added to the main quantity. The total weight of polyindene was 13.9 gm. It was purified by precipitation with alcohol from benzene solution; m.p., 133-137° C. Mol. wt., 624, 635. C, 92.7; H, 6.84%.

Distillate. By distillation at 2 mm. four fractions as follows were obtained. *1.* 4.6 gm. indene boiling at 35-40° C. *2.* 3.77 gm. of a golden yellow oil boiling at 151-160° C. 2 mm. It corresponded to diindene. On cooling it began to crystallize and after two recrystallizations from acetic acid melted at 57° C. *3.* 4.2 gm. of a viscous orange colored oil, boiling at 226-235° C. After cooling it was resin-like in appearance and after recrystallization from a large volume of boiling absolute alcohol melted at 69-71° C. Mol. wt. before recrystallization, 293. Mol. wt. after recrystallization, 339, 343 (calcd. for $(C_9H_8)_3$, 348). *4.* The residue in the flask was taken up in 25 cc. of chloroform; 0.6 gm. of insoluble material was separated, and the polymer was precipitated with 100 cc. of alcohol. Wt., 1.8 gm., m.p. after two precipitations from benzene, 108-110° C. Mol. wt., 493, 506. C, 92.90; H, 6.86%.

Thermo-polymer

Indene (115 gm.) was heated for 14 days at 177° C. in a sealed tube and the resulting orange colored resin was dissolved in chloroform. Precipitation was

brought about by the addition of alcohol and was repeated twice, using benzene as a solvent. The yield was 77.7 gm. of an amorphous white powder melting at 136-139° C. Mol. wt. 591, 608.

Seventy-five grams was subjected to pyrolytic distillation at 2 mm. over a period of 7.5 hr. The temperatures over which distillation occurred were as follows:— Bath, 164 to over 400°; distillate, 82 to 210° C. A yellow oil boiling at 82° C. began to come over when the bath temperature was 164° C. Distillation soon ceased, but, on raising the bath temperature to 195° C., a viscous orange colored oil came over at 168-193° C.

Residue. The residue in the distilling flask was treated with 200 cc. of chloroform and insoluble material (4.6 gm.) removed. Alcohol (800 cc.) was added and yielded a precipitate of 14.9 gm. M.p. 121-124° C. Mol. wt., 567. On concentrating the mother liquor to 50 cc., a pasty precipitate was obtained. It was taken up in 250 cc. of hot acetic acid and precipitated as a yellow amorphous powder by the addition of about 2 cc. of water. Wt., 2.4 gm. M.p., 100-102° C. Mol. wt., 468, 434.

Distillate. The distillate, amounting to 49.9 gm., was separated into four fractions as follows by distillation at 2 mm. *1.* 15.8 gm. of indene boiling at 35-40° C. *2.* 14.7 gm. of a yellow oil boiling at 160-175° C. at about 4 mm. By dissolving it in acetic acid and cooling, crystals of diindene, m.p. 56-57° C. were obtained. *3.* 18.2 gm. of viscous, red oil boiling at 224-235° C. 2 mm. On cooling, the oil resinified, and after recrystallization from hot absolute alcohol melted at 70-72° C. Mol. wt., 367. *4.* The residue (1.2 gm.) in the distilling flask consisted mainly of truxene, m.p. 360° C.

Bromine Absorption of Some Low Polyindenes

The amount of bromine absorbed by a number of the di- and triindene and low polymers isolated in the pyrolytic decomposition experiments was determined by adding to the samples, dissolved in chloroform, a measured quantity of a 0.078N solution of bromine in chloroform, and, after 24 hr. standing in the dark, titrating excess bromine. For comparison, the bromine absorption of a sample of indene was determined by the same procedure. The results are given in Table VI.

Effect of Heat on Polyindenes

In these experiments temperatures, 177° and 197° C., below those necessary to effect pyrolytic distillation were applied over periods up to four weeks. Except in one case the samples of polyindene used were the "homogeneous" high and low polymeric fractions of molecular weights 2631 and 608 respectively, the preparation of which has already been described. The samples were heated in sealed tubes and after heating were extracted in a Soxhlet extractor with chloroform in order to free the material from insoluble decomposition products. The chloroform was removed—the last traces with a vacuum pump—and duplicate determinations made of the molecular weight. The results are given in Table IV.

TABLE VI

BROMINE ABSORPTION OF FRACTIONS FROM PYROLYTIC DECOMPOSITION EXPERIMENTS

Sample	Mol. wt. (mean)	Weight taken, gm.	Bromine solution added, cc.	0.1N thiosulphate for excess bromine, cc.	Bromine absorbed per mole, gm.
1. Blank			50.0	30.05	
2. Indene (b.p. 181-2°)	116	0.1022	50.0	21.6	158
3. Diindene fraction in distillate, Expt. I	224	0.3332	50.0	9.8	156.8
4. Diindene fraction in distillate, Expt. II	222	0.2375	49.9	19.1	148.1
5. Triindene fraction in distillate, Expt. I	339	0.2250	40.1	17.9	161.5
6. Crude triindene fraction (Fraction 3), Expt. II	280	0.3916	50.0	16.3	147.2
7. Polyindene, m.p. 106-9° C., Expt. I	549	0.6441	49.8	14.7	175.0
8. Polyindene, m.p. 178-182° C., Expt. II	892	0.6885	50.0	21.4	182.3
9. Polyindene, m.p. 120-122° C., Expt. II	474	0.7495	49.8	5.9	166.6

DIINDENE

Preparation

The method of preparation described by Weissgerber (9) gave only a 10% yield of diindene after 24 hr. heating. The heated mixture of indene and acetic acid turned yellow but did not separate into two layers.

Using phosphoric acid as a condensing agent (9), 100 gm. of indene gave 60 gm. of a diindene fraction boiling at 225-235° C./20 mm. and yielding on crystallization from glacial acetic acid 44 gm. of diindene melting at 55-57° C.

The use of hydrochloric acid as the polymerizing agent (5) gave diindene in best yield and in purest form. A mixture of 100 gm. of indene, 100 cc. of a 23% hydrochloric acid solution and some pumice stone was refluxed for 10 hr. and the reddish oil was separated, washed with sodium carbonate solution, dried, and fractionated at 2 mm. About 10 gm. of indene (b.p. 79-82° C./20 mm.) was recovered. The second fraction, collected at 228-238° C. was the main one. A third fraction, boiling at 260-300° C., amounted to less than 10 gm. The second fraction was redistilled at 12 mm. and a yellow oil obtained at 205-212° C., which, after recrystallization from glacial acetic acid, gave 63 gm. of diindene melting at 56-57° C.

Pyrolytic Distillation

A fraction representing diindene obtained by the pyrolytic distillation of a sample of polyindene (Experiment II) was distilled at ordinary pressure, using a metal bath. The material was a golden yellow oil with a molecular weight of 222. The dimer did not begin to depolymerize and yield distillate until the bath temperature reached 340° C. Distillate (7.7 gm.) came over at 179-189°, while the bath was kept at 340-350° C. It had the odor of indene and when

redistilled boiled for the most part at 181-182.5° C. The residue in the distilling flask was treated with 25 cc. of ether and 0.6 gm. of a polyindene obtained on adding absolute alcohol to the ethereal solution. M.p., 97-99° C. Mol. wt., 440, 454. C, 93.05; H, 6.80% (calcd. for $(C_9H_8)_n$: 93.10, 6.90%).

The portion of the residue which failed to dissolve in ether was extracted with 50 cc. of hot benzene. From the benzene solution absolute alcohol precipitated 1.4 gm. of a yellow powder, melting at 208-210° C., which apparently was not polyindene, as it was only sparingly soluble in cold benzene. The residue (0.6 gm.) left from the hot benzene extraction melted at about 355° C. and was considered to be truxene.

Polymerization

By Sulphuric Acid. Ten grams of diindene (m.p. 57° C.) in 25 cc. benzene was treated with 25 cc. of concentrated sulphuric acid. There was a slight evolution of heat and a red product was formed. After three hours' standing, 150 cc. of absolute alcohol was added. The precipitate, purified by three reprecipitations from benzene, consisted of 6.3 gm. of a yellow amorphous powder melting at 96-98° C. C, 93.10; H, 6.82%. The product was separated into three fractions as follows:— It was treated with 100 cc. of hot glacial acetic acid, in which most of it dissolved. The residue was taken up in benzene and thrown out by pouring the solution into a large volume of alcohol. This gave Fraction 1 (Mol. wt. 675). Addition of a small amount of water to the acetic acid solution gave Fraction 2 (Mol. wt. 615), and addition of more water, Fraction 3 (Mol. wt. 464).

By Antimony Pentachloride. To 10 gm. of crystalline diindene in 25 cc. of chloroform 2 cc. of a 20% solution of antimony pentachloride in chloroform was added. The temperature rose immediately and a deep violet color appeared. After 24 hr. standing 100 cc. of absolute alcohol was added. The precipitate obtained was purified by throwing it out three times from benzene solution by alcohol. The polymeric product was 2.5 gm. of a dark yellow powder melting at 208-211° C. Mol. wt. 1246, 1219. C, 92.6; H, [illegible]%. On concentrating the mother liquors to 25 cc., unchanged diindene separated out in large crystals, the amount recovered being 6.3 gm., m.p. 55-57° C.

By Heat. Diindene (8.3 gm., m.p. 57° C.) was heated in a sealed tube for 11 days at 179-180° C. Examination of the yellow viscous oil which resulted failed to show the presence of any polymerized material. The molecular weight (226, 229, in two determinations) agreed with that of diindene. On dissolving the oil in 20 cc. benzene and adding 100 cc. of alcohol, no precipitate formed, and, on then evaporating the liquid to one-third of its volume, there was still no separation.

Samples of 6-8 gm. each of diindene which had been recrystallized twice from acetic acid were heated in sealed tubes at 214° and 258° C. for periods up to 21 days with results which are summarized in Table V. The samples assumed the appearance, according to the extent of heating, of an orange colored resin or gel. The contents of the tubes were dissolved in benzene, and, after filtering to remove any suspended matter, the solutions were poured into four volumes

of alcohol and the pale yellow polymeric product separated. If less than 35-40% of the diindene had undergone polymerization, the precipitate was soft and sticky. In such cases it was purified by dissolving in a large volume of hot glacial acetic acid and adding 1 or 2 cc. of water. This gave an amorphous powder which could be freed from traces of solvent and dried thoroughly. Molecular-weight determinations were made in duplicate. In one case, *viz.*, the polymer isolated after heating diindene for 15 days at 214° C., the product was separated into two fractions, for which data are as follows:- 1st fraction: mol. wt., 460. M.p., 100-102°. C, 93.2; H, 6.74%. 2nd fraction: mol. wt., 342. M.p. 69-71° C. C, 92.85; H, 6.62%. Analysis of polymer from 21 days' heating at 238° C.:– C, 92.85; H, 6.91%.

Polymerization of a Mixture of Indene and Diindene

I. A mixture of 5 gm. of indene and 5 gm. of diindene was heated for 15 days at 177° C. in a sealed tube. The soft yellow resin produced was distilled at 2 mm. by a water bath at 50-60° C. Unchanged indene (3.8 gm.) came over. The residue was dissolved in chloroform, and absolute alcohol was added. This yielded 3.3 gm. of polymer as a precipitate. The product was purified by solution in 150 cc. of hot absolute alcohol, from which it separated as white solid on cooling. M.p., 68-70° C. Mol. wt., 356, 345 (calcd. for $(C_9H_8)_3$:348). By evaporation of the mother liquor from which the polymer had been precipitated, unchanged diindene was recovered as a yellow oil to the extent of 2.8 gm. Mol. wt., 234.

II. A mixture of 3 gm. of indene and 3 gm. of diindene was heated for $5\frac{1}{2}$ days at 197° C. in a sealed tube and the resulting resin treated as in the preceding experiment. There was recovered 2.8 gm. of trimer (m.p. after recrystallization from a large volume of alcohol, 68-70° C. Mol. wt., 364, 348) and 1.2 gm. of unchanged dimer (mol. wt., 234).

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STUDIES IN URETHANES

V. NEW ACYL DI-URETHANES AND THEIR REACTIONS WITH AMMONIA AND AMINES[1]

By Steward Basterfield[2], C. Vernon Wilson[3] and Margaret E. Greig[4]

Abstract

Previous studies have shown that oxalyl-, malonyl- and phthalyldi-urethanes are decomposed readily by ammonia and primary amines with the loss of one or both urethane groups and the production of the amides of the dibasic acids, or of urethane-amides, and ureido-amides. Acyldi-ureas were not formed as might have been expected.

The present study deals mainly with the preparation and properties of succinyl-, glutaryl-, and adipyldi-urethanes. While with ammonia and ethylamine these yield amides, with aniline they give the acyldi-(phenylureas), which are decomposed by further action of the base into acylanilides, and diphenylurea. The mode of decomposition is strongly influenced by the nature of the acyl radical as well as that of the reacting base. The results may be provisionally generalized in the statement that, with a given base, the tendency of a di-urethane to yield the acid amide is more pronounced the stronger the acid from which the radical is derived, while with a given di-urethane the tendency to amide formation is more pronounced the stronger the reacting base. The formation of acyldi-ureas becomes evident when the di-urethane contains the radical of an acid with an ionization constant of the order of 10^{-5} or less.

Introduction

In a previous paper from this laboratory (5) it was pointed out that oxalyl-, malonyl-, and phthalyldi-urethanes in their reactions with ammonia and primary amines showed a definite tendency to lose urethane groups by ammonolysis, yielding amides of the stronger acids in preference to acyldicarbamides. With a weak base such as aniline it was found possible by regulation of temperature and time to isolate intermediate products of decomposition in which one urethane group was still present as such or had been converted to a phenyl-carbamide group. These intermediate products were readily converted by further heating with aniline into the anilides of oxalic, malonic, and phthalic acids, and diphenylurea.

As a further contribution to this study, and with a view to securing a broader basis for generalization, it was decided to prepare di-urethanes of succinic, glutaric and adipic acids, and investigate their behavior with ammonia and primary bases. It was also planned to study the effect on this type of reaction of the introduction of a variety of substituting groups into malonyl-, succinyl-, and phthalyldi-urethanes, and for this purpose the following acids were selected for preliminary study: bromomalonic, phenyl- and *p*-nitrophenyl-malonic,

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Contribution from the Chemical Laboratory, University of Saskatchewan, Saskatoon, Canada. This paper is based mainly on a thesis presented by C. Vernon Wilson in partial fulfilment of the requirements for the degree of M.Sc. at the University of Saskatchewan. A minor part is from a thesis presented by Margaret E. Greig for the degree of M.A. at the same university.

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phenylsuccinic and 3-nitrophthalic acids. Bromomalonic and 3-nitrophthalic acids have ionization constants in the same region as that of oxalic acid, and phenylsuccinic acid has an ionization constant in the region 10^{-4}, between those of malonic and succinic acids. Phenylmalonic and *p*-nitrophenylmalonic acids were selected in order to obtain, if possible, additional evidence of the influence of a nitro group on the decomposition of the respective di-urethanes.

In the preparation of substituted di-urethanes certain difficulties were encountered which have been only partially overcome. The di-urethanes of bromomalonic, phenylmalonic, and 3-nitrophthalic acids have been obtained in small yields, but *p*-nitrophenylmalonic acid and its di-urethane have not yet been prepared. A special study of this problem is in progress in this laboratory at the present time. In this paper are described the preparation and decomposition of succinyl-, glutaryl-, adipyl-, and bromomalonyldi-urethanes, and the preparation of 3-nitrophthalyl-, and phenylmalonyldi-urethanes. The decomposition of these last two compounds has not yet been studied.

Preparation of Di-urethanes

In the earlier work on the preparation of di-urethanes carried out in this laboratory (5), the chlorides of the dibasic acids were made to react with urethane with good results. In the present study the method of Backes, West and Whiteley (1) has been used for the most part in order to avoid the necessity of preparing the acid chlorides. The method consists in warming the dibasic acids with urethane and phosphorus oxychloride, the di-urethane being precipitated on the addition of water to the reaction mixture. The results have been excellent in some instances, but in others the required product was not obtained. For example, when dibromomalonic and dibromosuccinic acids were treated with urethane and phosphorus oxychloride, only black tarry products were obtained, while with diethylmalonic acid, a good yield of allophanic ester was obtained. The production of allophanic ester had previously been observed during an attempt to make oxaly'di-urethane by this method (4), and in the preparation of 3-nitrophthalyldi-urethane a considerable quantity of the ester appeared as a constant by-product.

It is well known that the action of certain acid chlorides on urethane gives rise to allophanic ester. Thionyl chloride (11) and carbonyl chloride (9) have both been reported as acting in this way. The latter will, however, in presence of pyridine, react smoothly with urethane to give carbonyl di-urethane (2, 7). During the present study it was observed that phosphorus oxychloride and urethane heated together at 60° to 70° C. for three or four days, gave a small amount of allophanic ester but when, for example, diethylmalonic acid was added, a much more vigorous reaction took place and a much larger amount of the ester was produced. No diethylmalonyldi-urethane was isolated.

For the preparation of phenylmalonyldi-urethane, phenylmalonic acid was synthesized from phenyl acetonitrile by the method of Rising and Stieglitz (10), based on the studies of Wislicenus (12). When the same method was applied to the production of *p*-nitrophenylmalonic acid the synthesis failed. In the

first stage of the work, methyl *p*-nitrophenylacetate was obtained in good yield, but this compound failed to condense with methyl oxalate to give the methyl *p*-nitro-phenyloxalacetate, the necessary precursor of methyl *p*-nitrophenylmalonate. Other methods for the preparation of this compound are therefore being investigated.

Action of Ammonia and Primary Amines on Di-urethanes

With concentrated aqueous ammonia at room temperature, succinyl-, glutaryl-, and adipyldi-urethanes are converted into amides apparently by hydrolysis of carbethoxy groups, since no urethane was detected in the reaction mixture. With alcoholic ammonia, the di-urethanes underwent no appreciable change. These results are noteworthy by comparison with the behavior of the di-urethanes of the much stronger oxalic and malonic acids which undergo ammonolysis with loss of urethane groups (5).

With ethylamine in either aqueous or alcoholic solution at room temperature, the three di-urethanes were found to be easily resolved into diethyl amides and urethane. It is interesting and significant that with this, the most basic reagent used, the five di-urethanes of the acids of the oxalic series are decisively decomposed into diethyl amides and urethane. Brom-malonyldi-urethane with similar treatment yielded a small quantity of the diethyl amide, and a large quantity of pink gummy material which evolved ethylamine on being warmed with alkali.

With aniline reactions were carried on at temperatures ranging from 100° to 210° C., and the interesting fact emerged that succinyl-, glutaryl-, and adipyl-di-urethanes all give acyl di-(phenylureas), which on being further heated with aniline, are decomposed into the acyl anilides and carbanilide (diphenylurea). From succinyldi-urethane there was also obtained a phenylureido-urethane of the structure A, evidently representing the first phase of the reaction since it was rapidly converted into the succinyldi-(phenylurea) on being warmed with aniline. Succinyldi-(phenylurea) undergoes much more rapid decomposition into the anilide and diphenylurea than do the di-ureas of glutaric and adipic acids. Evidence of the existence of intermediate products formed in the conversion of the acyl di-ureas to anilides was also obtained, but only from the decomposition of adipyldi-(phenylurea) was a definite product isolated in small quantity, the analysis of which corresponded to that of the phenylureidoanilide. The isolation of pure substances from mixtures of anilides and ureas has proved tedious and difficult, owing to the similarity in their solubilities and the tendency to form mixtures of constant melting point.

$$\begin{array}{l} CH_2.\ CO.\ NH.\ COOC_2H_5 \\ | \\ CH_2.\ CO.\ NH.\ CO.\ NHC_6H_5 \end{array}$$

A

$$(CH_2)_4 \begin{cases} CO.\ NHC_6H_5 \\ CO.\ NH.\ CO.\ NHC_6H_5 \end{cases}$$

It would appear from the results of the reactions with aniline of the di-urethanes of the oxalic acid series that a point is reached at succinic acid, where the acyl di-(phenylurea) is sufficiently stable to be formed. Reference

to the table of ionization constants of the acids shows a rather large change in the strength of the acids at this point, malonic acid having a constant of the order of 10^{-3}, while the constants for succinic, glutaric and adipic acids, are all of the order of 10^{-5}.

In the earlier paper (5) it was suggested that the nature of the acyl radical influenced the decomposition of the di-urethanes in the sense that there was a definite tendency towards the formation of the amides of the stronger acids in preference to carbamides; that probably the greater free energy of formation of the amides of the stronger acids caused this reaction to predominate. There is a converse aspect of these reactions to that already noted, related to the relative strengths of the bases used as reagents. The twofold aspect of the situation may be expressed as follows:– With a given ammonia base the tendency of an acyl di-urethane to decompose into the acyl amide and urethane is more pronounced the stronger the acid from which the radical is derived; with a given acyl di-urethane the tendency to this type of decomposition is more pronounced the stronger the reacting base.

TABLE I

Ionization constants of dibasic acids (8)

Acid	K (first hydrogen)	Acid	K (first hydrogen)
Carbonic	3.0×10^{-7}	Adipic	3.7×10^{-5}
Oxalic	3.8×10^{-2}	Phthalic	1.2×10^{-3}
Malonic	1.6×10^{-3}	3-Nitrophthalic	1.31×10^{-2}
Succinic	6.6×10^{-5}	Bromomalonic	Unrecorded
Glutaric	4.7×10^{-5}	Phenymalonic	Unrecorded

Experimental

Preparation of Acyl Di-urethanes

Succinyldi-urethane $(CH_2.CONHCOOC_2H_5)_2$. A mixture of succinyl chloride and urethane (molecular proportions 1:2) was allowed to stand for three weeks and then heated to 60° C. for three days. Recrystallized from alcohol, succinyl di-urethane was finally obtained in pure white flaky crystals, m.p., 155° C., yield, 25%. Calcd. for $C_{10}H_{16}N_2O_6$; N, 10.68. Found: N, 10.50, 10.56%.

A mixture of succinic acid, phosphorus oxychloride, and urethane (molecular proportions 1:1:2), after standing for ten weeks was treated with water and gave a 38% yield of the di-urethane. Another mixture after standing for nine weeks was heated to 60°-70° C. for 24 hr. About the same yield of the di-urethane was obtained. This quantity was the maximum obtained in any experiment. The various mixtures, if heated immediately after being made, failed to give crystalline products.

Glutaryldi-urethane $(CH_2)_3(CONHCOOC_2H_5)_2$. Glutaric acid, phosphorus oxychloride, and urethane were mixed and allowed to stand under a condenser for three or four hours. The mixture became solid and was then warmed for

three hours at 60-70° C. The reaction mixture was poured into water, and the precipitated solid was separated and recrystallized from ethyl alcohol. It formed masses of silky lath-like crystals, m.p. 192° C., yield 75%. Calcd. for $C_{11}H_{18}N_2O_6$; N, 10.21. Found: N, 9.86, 10.02%.

Adipyldi-urethane $(CH_2)_4(CONHCOOC_2H_5)_2$. A mixture of adipic acid, phosphorus oxychloride and urethane was allowed to stand at room temperature until it solidified. It was then heated for two or three hours at 60-70° C. and treated with water. The solid material was separated and recrystallized from alcohol. It formed white flaky crystals, melting at 174° C., yield 60%. Calcd. for $C_{12}H_{20}N_2O_6$; N, 9.79. Found; N, 9.76, 9.59%. If the reaction mixture was heated initially it became dark and the yield of di-urethane was much reduced.

Bromomalonyldi-urethane $CHBr(CONHCOOC_2H_5)_2$. This di-urethane has been previously reported by Backes, West and Whitely (1). It was readily obtained by brominating malonyldi-urethane in chloroform solution.

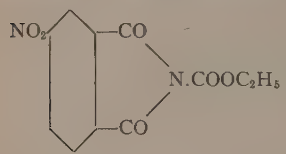
Phenylmalonyldi-urethane $C_6H_5CH(CONHCOOC_2H_5)_2$. Methyl phenylmalonate, prepared by the method of Rising and Stieglitz (10) was hydrolyzed, as recommended by Wislicenus (12), by warming in methyl alcohol containing sodium methoxide and a limited amount of water. The sodium salt of phenylmalonic acid that separated was treated with dilute sulphuric acid and cooled in ice. Some of the organic acid crystallized out and the rest was extracted with ether. Even under these conditions some of the phenylmalonic acid was converted into phenylacetic acid. Hydrolysis in ordinary aqueous alkali yielded only phenylacetic acid. The acid melted at 153° C.

A mixture of 6 gm. of phenylmalonic acid, 6 gm. of urethane, and 5.1. gm. of phosphorus oxychloride was heated under a condenser for 12 hr. at 65-70° C. The yellow gummy mixture was treated with water and allowed to stand overnight. A white solid product was obtained which was crytallized from boiling alcohol. The substance melted at 154° C.; yield, 3 gm. Calcd. for $C_{15}H_{18}N_2O_6$; N, 8.69. Found: N, 8.89, 8.92%.

3-Nitrophthalyldi-urethane $NO_2.C_6H_3(CONHCOOC_2H_5)_2$. A mixture of 10.4 gm. of 3-nitrophthalic acid, 9 gm. of urethane and 7.7 gm. of phosphorus oxychloride was warmed for two days at 60-70° C. Water was added and the insoluble residue was separated and dissolved in hot alcohol. The first crop of crystals proved to be almost pure allophanic ester. The filtrate was diluted with water and the precipitated solid was dried, washed with ether and recrystallized from a mixture of alcohol and ether. The melting point was not definite; softening and decomposition took place at 115-122° C. (Phthalyldi-urethane showed somewhat similar behavior (5).) The yield was poor. Calcd. for $C_{14}H_{15}N_3O_8$; N, 11.89. Found: N,12.23, 12.17, 12.05%.

In order to obtain, if possible, a larger yield of the di-urethane the 3-nitrophthalic acid was converted into the acid chloride by the method of Chambers (6). A mixture of the acid chloride, urethane, and pyridine (molecular proportions 1:2:2) was heated in benzene solution on a water bath for two days. The solid which separated on cooling was washed with ether and benzene and found to be identical with the product decomposing at 115-122° C. obtained

by the first method. Attempts to recrystallize the substance from hot solvents only brought about decomposition. When it was dissolved, for example, in boiling alcohol, a substance melting at 215° C. separated from the cooled solution. This was apparently 3-nitrophthalimide. From hot benzene solution a different substance was obtained. It melted at 95° C. and had a nitrogen content of 10.40%. It was presumably 3-nitro-carbethoxy-phthalimide, which contains 10.60% nitrogen.



In the study of phthalyldi-(methyl iso-urea) by Basterfield and Powell (3) a similar phenomenon was encountered. This compound is unstable in hot solvents and appears to lose a molecule of iso-urea readily.

Decomposition of Acyl Di-urethanes by Ammonia and Amines

Succinyldi-urethane

Action of ammonia. Four grams of succinyldi-urethane was treated with concentrated aqueous ammonia at room temperature. After the mixture had stood several days a heavy white precipitate was filtered off and identified as succinamide, m.p. 240° C., by a mixed melting-point determination with a known sample. Since there was no urethane in the filtrate, the reaction was apparently one of hydrolysis of the carbethoxy groups.

Action of ethylamine. Aqueous ethylamine (17%) was added to succinyldi-urethane and the mixture allowed to stand at room temperature for a day or two. The clear solution was evaporated almost to dryness and the residue treated with ether. A white crystalline substance separated and was identified as diethyl-succinamide, m.p. 200° C. Calcd. for $C_8H_{16}N_2O_2$; N, 16.28. Found: N, 16.16, 16.12%. The ethereal filtrate on evaporation yielded urethane, identified by a mixed melting-point determination with a known sample. Alcoholic ethylamine (14%) and succinyldi-urethane yielded the same products.

Action of aniline. Succinyldi-urethane and aniline (molecular proportions 1:6) were heated together at 100° and 120° C. for several hours. Very little decomposition occurred, most of the di-urethane being recovered unchanged. Mixtures of the di-urethane and aniline in the same proportion heated for two hours at 150° C. gave mainly succinanilide and diphenylurea, both of which were identified by comparison with known samples. The substances were easily separated by crystallization from hot alcohol or by extraction of the diphenylurea with ether.

In order to obtain possible intermediate products mixtures were heated for one hour only, and the semi-solid product treated with hot alcohol. An insoluble portion was separated by filtration, washed thoroughly with alcohol and dried. It was only very slightly soluble in hot alcohol and was recrystallized with difficulty. It formed fine sandy crystals melting at 236° C., and was apparently succinyldi-(phenylurea); yield, 66%. Calcd. for $C_{18}H_{18}N_4O_4$; N,15.7. Found: N, 15.31, 15.56%.

The alcohol-soluble portion of the original mixture of reaction products was separated by crystallization from alcohol into two portions. One, melting at 156° C., was found to be unchanged succinyldi-urethane, the other, melting at 163° C., was a new compound. Found: N, 13.94, 13.80%.

The two substances that could be formed in the reaction are indicated below with their respective nitrogen contents.

$$
\begin{array}{ll}
 & \mathrm{CO.\ NH.\ COOC_2H_5} \\
 & \quad | \\
\mathrm{(I)} & \mathrm{(CH_2)_2} \\
 & \quad | \\
 & \mathrm{CO.\ NH.\ CO.\ NHC_6H_5} \\
 & \\
 & \quad \mathrm{N,\ 13.67\%}
\end{array}
\qquad\qquad
\begin{array}{ll}
 & \mathrm{CO.NHC_6H_5} \\
 & \quad | \\
\mathrm{(II)} & \mathrm{(CH_2)_2} \\
 & \quad | \\
 & \mathrm{CO.\ NH.\ CO.\ NHC_6H_5} \\
 & \\
 & \quad \mathrm{N,\ 13.50\%}
\end{array}
$$

The substance melting at 163° C. was warmed with aniline at 150° C. for three-quarters of an hour, and was converted completely into succinyldi-(phenylurea). Since it is impossible for the di-urea to be formed from (II), it follows that the compound melting at 163° C. is (I), phenyl-ureido-succinyl-urethane, and represents the first stage in the formation of the di-urea. The latter was in turn rapidly decomposed by heating with aniline for one hour into succinanilide and diphenylurea. This accounts for the fact that it was not isolated from the reaction mixture that was heated at 150° C. for two hours.

It is of interest to note that in the decomposition of malonyldi-urethane with aniline at 150° C., a compound corresponding to (II) was obtained (5), but no di-urea. In this reaction one urethane group is probably split off while the other is transformed to a phenyl-urea group.

Mixtures of di-urethane and aniline heated at 190° C. gave quantitative yields of succinanilide and diphenylurea.

Glutaryldi-urethane

Action of ammonia. Glutaryldi-urethane was mixed with concentrated aqueous ammonia and allowed to stand at room temperature for a few days. The white product that separated was identified as glutaramide, m.p. 176° C. A small quantity of an unidentified product melting at 265° was also present.

Action of ethylamine. Fifty cc. of aqueous ethylamine (18%) was added to 4 gm. of glutaryldi-urethane and the mixture allowed to stand at room temperature. Solution was complete in several hours and no solid product was precipitated on further standing. The clear liquid was evaporated to dryness and the residue extracted with ether several times. The ether insoluble residue, m.p. 144° C , was identified as diethylglutaramide. Calcd. for $C_9H_{18}N_2O_2$; N, 15.05. Found: N, 14.90, 14.94%. When the ether extract was evaporated an oil was left which solidified in a desiccator. It was purified and identified as urethane.

Alcoholic ethylamine (14%) decomposed glutaryldi-urethane into the same products as described in the previous section. The di-urethane reacted more slowly with the alcoholic solution than with the aqueous one. Other experiments in this laboratory, to be reported in another paper, confirm the sugges-

tion that the ammonolytic action of ethylamine is stronger in water than in alcohol solution.

Action of aniline. Three grams of glutaryldi-urethane and 6.2 gm. of aniline was heated at 150° C. for two hours. The di-urethane first dissolved and in a short time a solid began to separate from the reaction mixture.

When the mixture was cool, the semi-solid mass was extracted with cold ether to remove unchanged aniline. The residue was crystallized from boiling alcohol in which it was only slightly soluble. The substance, both before and after crystallization, melted at 219-220° C. It was undoubtedly glutaryldi-(phenylurea). Calcd. for $C_{19}H_{20}N_4O_4$; N, 15.22. Found: N, 14.62, 14.91%. No other compound was detected. That the glutaryldi-(phenylurea) is much more stable than the succinyl compound is evident from this result, and this is more clearly indicated by its slow reaction with aniline at 190°-200° C.

A mixture of di-urethane and aniline (molecular proportions 1:6) was heated at 190°-200° C. for three hours. From the clear solution a solid separated in the course of 15 to 20 min., but was redissolved as the heating was continued. This phenomenon was due to the glutaryldi-(phenylurea) first formed being slowly decomposed. At the end of three hours about 50% of the di-urea was still present. A small quantity of diphenylurea was found in the reaction mixture but pure glutaranilide, the other product of decomposition, was not isolated. This was probably due to the presence of the intermediate ureido-anilide, from which continued recrystallization failed to separate it, and also to the tendency observed both with malonanilide (5) and adipanilide to form constant melting-point mixtures with diphenylurea.

In another experiment heating at 200° C. for six hours effectively decomposed the di-urea, but while a considerable amount of diphenylurea was isolated, glutaranilide was not definitely identified in the residue.

Adipyldi-urethane

On account of the cheapness and availability of adipic acid, a more exhaustive study was made of the reactions of adipyldi-urethane than of those of the glutaryl compound.

Action of ammonia. Four grams of adipyldi-urethane was allowed to stand in concentrated aqueous ammonia for four days at room temperature. By this time the mixture was almost solid. It was drained on a suction filter and repeatedly extracted with hot alcohol. From the alcoholic extracts, a white solid separated. It melted at 217° C. and was apparently adipamide. Calcd. for $C_6H_{12}N_2O_2$; N, 19.44. Found: N, 19.54%.

The alcohol-insoluble residue did not melt below 300° C. It dissolved in boiling water and separated as a gel when the solution was cooled. These facts suggest that it is a compound of high molecular weight. Its content of nitrogen, 15.25%, does not agree with that of any simple decomposition product of the di-urethane.

Alcoholic ammonia had no appreciable action on adipyldi-urethane even when mixtures were allowed to stand for several weeks at room temperature. The di-urethane was recovered largely unchanged.

Reactions with ammonia at higher temperatures and under pressure will be examined later.

Action of ethylamine. Forty cc. of aqueous ethylamine (17%) was added to 4 gm. of adipyldi-urethane and the mixture allowed to stand for two or three days. The clear solution was evaporated and the residue extracted with ether. The insoluble portion was recrystallized from alcohol. It formed leaf-like crystals melting at 164° C., and was identified as diethyl-adipamide. Calcd. for $C_{10}H_{20}N_2O_2$; N, 14.00. Found: N, 13.61, 13.74%. The ether extract yielded urethane.

Alcoholic ethylamine (14%) decomposed the di-urethane in a similar manner but more slowly.

Action of aniline. There was no appreciable action at 100° and 120° C.

Mixtures of adipyldi-urethane and aniline (molecular proportions 1:6) heated for two hours at 150° C. yielded mainly a product melting at 225° C. The solid product of the reaction was thoroughly extracted with ether to remove unchanged aniline and also diphenyl urea. The residue was practically insoluble in boiling alcohol and was analyzed after thorough extraction with this solvent. It was apparently adipyldi-(phenylurea). Calcd. for $C_{20}H_{22}N_4O_4$; N, 14.65. Found: N, 14.41, 14.47%. No diphenylurea was found in the ether or alcohol extract.

The main product after two hours' heating at 190° C. was again adipyldi-(phenylurea). Diphenylurea was present in small quantity indicating that some decomposition of the di-urea had taken place. From the ether extract of the reaction mixture a small quantity of a substance melting at 172° C. was also obtained. From the analysis it was judged to be phenyl-ureido-adipanilide $C_6H_5NHCONH.CO(CH_2)_4.CONHC_6H_5$. Calcd. for $C_{19}H_{21}N_3O_3$; N, 12.38. Found: N, 12.15%. No adipanilide was found.

The di-urea was formed rapidly at this temperature, a considerable quantity of solid separating out after 20 min. heating. If the reaction was stopped at this point, the product was found to be entirely the di-urea without any trace of diphenylurea or anilide present.

In order to obtain a more complete decomposition of the di-urea, it was heated with aniline for 3 hr. at 200°-210° C. In an actual experiment under these conditions, 4.6 gm. of adipyldi-(phenylurea) gave 5.3 gm. of product entirely soluble in hot alcohol. This product was resolved into diphenylurea and a substance which after repeated recrystallization melted at 208° C. Found: N, 10.69, 10.53, 10.30%. There is no possible decomposition product of the di-urea having such a nitrogen content, and it was judged to be a constant melting-point mixture of adipanilide and diphenylurea. The nitrogen contents of these are 9.46 and 13.21% respectively. To obtain larger quantities of this mixture for resolution, 10 gm. adipyldi-urethane was heated with excess of aniline at 210-220° C. for 20 hr. Three grams of the substance melting at 208° C. was obtained. It was extracted twice with 125 cc. of ether. The extract contained impure diphenylurea as expected, and the melting point of the ether-insoluble residue had risen to 238° C. This residue was identified as adipanilide by a mixed melting-point determination with a known sample.

From the alcoholic mother liquor after the crystallization of the constant melting-point mixture a product was obtained melting at 165°-175° C. It was judged to be a mixture since its nitrogen content of 14.0% did not seem to agree with that of any possible decomposition product of the di-urea. It was not resolved.

Bromomalonyldi-urethane

Action of ethylamine. The di-urethane was treated in the usual way with aqueous ethylamine (17-18%), and allowed to stand for three days at room temperature. A small quantity of allophanic ester, m.p. 192° C., separated and was removed by filtration. The filtrate was evaporated at room temperature until a white crystalline product separated. This was identified as diethyl-bromomalonamide, m.p. 160° C. The residue obtained by complete evaporation was a pink gum, which probably contained more of the amide, since on being warmed with alkali it evolved ethylamine.

The appearance of allophanic ester as a product of this reaction is peculiar, but it points to urethane having been split off in the first place, the latter then being converted to the allophanic ester.

Action of aniline. A mixture of the usual molecular proportions was heated for two hours at 150° C. The solid product was washed with ether. The residue did not melt definitely, but on being crystallized from alcohol, it separated into diphenylurea and a substance that did not melt below 300° C. Found: N, 13.73, 13.90%.

Two grams of bromomalonyldi-urethane and excess of aniline heated for two hours at 190° C. gave a 40% yield of diphenylurea and 0.8 gm. of the same substance obtained at 150° C. This substance contained no bromine, indicating that the halogen had reacted with aniline at these temperatures. It was probably a ureido-anilide of the structure B. N calculated for the above is 14.04%. The formation of this substance as the principal product agrees with the 40% yield of diphenylurea. The displacement of bromine by the C_6H_5NH-residue alters the nature of the acid radical, so that it is impossible to determine the effect on the course of the reaction of the presence of the halogen. The decomposition of this di-urethane is somewhat analogous to that of the unsubstituted malonyldi-urethane at 150° C., where a ureido-anilide was also formed (5).

```
              CO.NHC6H5
              |
C6H5NH—CH
              |
              CO.NHCONHC6H5
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B

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES. XXXV. POLYSACCHARIDE SYNTHESIS BY THE ACTION OF *ACETOBACTER XYLINUS* ON CARBOHYDRATES AND RELATED COMPOUNDS[1]

By H. L. A. Tarr[2] and Harold Hibbert[3]

Abstract

The action of *Acetobacter xylinus*, when cultivated in nutrient solutions containing certain carbohydrates or polyhydric alcohols, in forming surface polysaccharide membranes has been confirmed. The nutrient substrate necessary for the effective synthesis of nitrogen and ash-free polysaccharides from such carbon compounds has been carefully determined, and the necessity for the presence of a small amount of ethyl alcohol definitely proved. This type of synthesis appears to be specific for this organism, in that only the hexoses, their anhydrides and compounds which presumably yield hexoses as a result of the bacterial action, give rise to polysaccharide formation. When the carbon compound used is not a free hexose, nor one apparently capable of conversion into a hexose, as in the case of pentoses such as arabinose and xylose, glycols, polyglycols and erythritol, no membrane formation takes place. On the other hand mannitol, which is known to undergo oxidation with the formation of fructose under the experimental conditions, gives rise to a high yield of a synthetic polysaccharide. Glycerol behaves similarly, due presumably to a primary oxidation to dihydroxy acetone and conversion of this to fructose, the latter then yielding the polysaccharide membrane. The introduction of a methyl group into glycerol and glucose, with the formation of α-methyl glycerol and α-methyl glucoside, respectively, inhibits polysaccharide formation. The product obtained from glucose was found to be very closely related to cellulose, if not identical with it (13). Galactose is much less reactive than glucose, while mannose appears to be relatively inactive with the strain of *A. xylinus* employed. The highest yield of the polysaccharide was obtained from fructose, a result presumably connected with the recognized fact that this bacterium forms little or no acid from this sugar.

Introduction

At the present time the constitution of cellulose remains uncertain, although many investigators regard it as a polymer of cellobiose anhydride. It is insoluble in water, alcohol, ether and dilute acids, and, on treatment with concentrated sulphuric acid and iodine or with chloriodide of zinc, gives a characteristic blue color, the so-called "cellulose reaction" employed by bacteriologists and botanists.

During his studies on *Bacterium aceti* Brown (4, 5) discovered an organism characterized by its ability to form extremely tough membranes when cultivated in suitable nutrient solutions containing carbohydrates. In some instances the membranes were 2.5 cm. thick, and in time sank to the bottom of the culture vessel, thus permitting a fresh layer to form. The causal organism exhibited decided morphological variations, the small rod-shaped bacteria, which predominated in young cultures, being replaced in older cultures by coccus forms. The optimum growth temperature was approximately 28° C.

[1] *Manuscript received March 17, 1931. Contribution from the laboratories of the Department of Bacteriology and the Department of Industrial and Cellulose Chemistry, McGill University, Montreal.*

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At 36° C. the organism was still alive, but ceased to multiply. The membranes thus formed yielded a reducing sugar upon hydrolysis with sulphuric acid. The membranes were moreover readily soluble in ammoniacal cupric hydroxide. Fructose was found to be the most fruitful source as raw material for the synthesis of the polysaccharide, mannitol coming next, and glucose last; ethyl alcohol, sucrose and starch, did not support the formation of the cellulose-like membrane. Brown (6) subsequently observed that the membrane prepared from fructose yielded a dextrorotatory sugar upon hydrolysis. He proposed the name *Bacterium xylinum* for this polysaccharide-forming organism.

Emmerling (9) showed that Bertrand's *Sorbose bacterium* (3) was identical with Brown's *Bacterium xylinum*. He obtained a strain of the organism from Bertrand and prepared the membranes by growing the bacterium on certain nutrient liquids. These membranes were only slightly soluble in ammoniacal cupric hydroxide, and contained between 2 and 3% of nitrogen. By treating these membranes with hydrochloric acid he obtained a crystalline product which resembled glucosamine hydrochloride, and therefore decided that the original membrane did not consist entirely of pure cellulose, but that a chitin-like substance was also present. Browne (7) found that a cellulose-like fermentation of sugar cane juice was of fairly frequent occurrence in Louisiana, and he described the causal organism as being probably identical with Brown's *B. xylinum*. He studied the cellulosic material obtained from sugar cane juice, and was unable to confirm Emmerling's results. He found that the dried membrane, after boiling with 10% caustic soda, contained only 0.2% nitrogen. Also only 1% was insoluble in ammoniacal cupric hydroxide solution, so that chitin could only be present in minute traces, if at all.

Beijerinck (1) observed that certain acetic acid forming bacteria other than *B. xylinum*, *e.g.*, *B. pasteurianum* and *B. rancens*, produced slimes giving the "cellulose reaction", and he believed them to be closely related structurally to the cell walls of the seeds of most leguminous plants and the ascus-bearing cell of the lichens. Van Wisselingh (18) also employed the "cellulose reaction" to determine the presence or absence of cellulose in bacterial cells. In some instances the bacteria were heated to 300° C. in glycerol prior to performing the test. Some 17 different organisms were examined, but only one, *viz.*, *B. xylinum*, gave a positive test. Migula (15) had previously claimed to have obtained a characteristic "cellulose reaction" by treating cultures of *Sarcina ventriculi* with iodine and sulphuric acid.

Henneberg (11, p. 212), describing *B. xylinoides*, an organism apparently very closely related to *B. xylinum*, states that a membrane, "xylinumhaut", is readily formed from yeast water to which arabinose, galactose, fructose, sucrose, maltose, lactose, mannitol etc. have been added. He also observes that, "Der Zucker ist nicht die Ursache der Ausbildung der "Xylinumhaut", da durch Zusatz von Zucker zur Weinessigmaische diese Hautart nicht hervorgerufen werden kann". If this statement is correct this organism must differ from *B. xylinum*, for Brown (5) showed definitely that certain sugars increased the formation of the cellulosic membranes when added to a yeast-water medium in which *B. xylinum* was cultivated.

Recently Eggert and Luft (8) studied *B. xylinum* membranes prepared for them by E. Schmidt, University of Munich, and found that dried preparations gave X-ray diagrams similar to those from β-cellulose. Ipatjef (14) has examined membranes obtained from the same source, but the amount of available material did not permit him to perform any conclusive tests. Neither of these two last-named investigators described the method of preparation of the material which they examined, nor the sugar source employed in its formation.

In no instance has a detailed study of the formation of cellulose by true bacteria (Eubacteriales (2)) been made, nor has there been any thorough chemical investigation of the polymerized product. The purpose of the present investigation has been, first, to study the method of formation of the cellulosic material by the action of *Acetobacter xylinus* (*B. xylinum*), and then to prepare sufficient pure material for an exhaustive chemical investigation of the compound.

Discussion of Results

A systematic series of experiments, conducted with a view to obtaining a culture medium which did not support visible growth until a suitable source of carbon was added, indicated that a solution containing 0.1% asparagine, 0.5% KH_2PO_4, 0.1% NaCl and 0.5% ethyl alcohol satisfied these requirements. The use of nitrogen compounds other than asparagine brought about two different results. The one type (peptone, yeast water) gave rise, in the absence of carbohydrates and related compounds, to pronounced zoogleal formation, for which reason their use was discarded. The second type (urea, inorganic ammonium salts) was found far less suitable than asparagine with respect to yield of synthetic polysaccharides from the carbon compounds employed.

The synthetic polysaccharide product obtained from the above medium may be assumed to have been formed entirely from the added carbon compound. Such solutions have been shown, after addition of a suitable carbohydrate or related compound, to yield a polysaccharide product readily freed from ash and nitrogen (13), thereby facilitating its chemical investigation. The amount of polysaccharide material formed by *A. xylinus* in such media reached a maximum in approximately ten days after inoculation. The observation of Herzog (12), that the addition of ethyl alcohol to the culture medium causes a very marked increase in the yield, was verified.

Exhaustive experiments, in which some twenty-five different carbon compounds and three different nutrient solutions were studied, showed that polysaccharide formation by *A. xylinus* occurs only with hexose sugars, their anhydrides, and substances which presumably can be converted readily into hexoses by this organism. Fructose was found to be the most suitable carbon compound for the formation of the polysaccharide pellicle, in this respect showing an activity much more pronounced than that of any of the aldohexoses. Furthermore polysaccharides containing anhydro-fructose units were apparently much more suitable for the synthesis of this polysaccharide than those containing anhydro-glucose units. Of the various other hexoses employed, glucose showed a marked activity, galactose considerably less, while mannose was relatively inert.

Neither rhamnose nor other pentoses, when inoculated with *A. xylinus* under the same conditions, gave rise to polysaccharide formation. β-Glucosan, glycerol chlorhydrin, ethylene glycol, tetra-ethylene and hexa-ethylene glycols were found to be equally inactive. The fact that α-methyl glucoside and α-methyl glycerol do not support polysaccharide formation shows the marked influence of a single methyl group in inhibiting membrane production since both glycerol and glucose gave a high yield.

Of the higher sugar alcohols no pellicle formation occurred in the case of erythritol (which is known to give *d*-erythrulose on oxidation (17, p. 166)). Mannitol gave a high yield, which is explained by the fact that it is known to undergo oxidation to fructose by this organism (17, p. 164).

It would appear from these results that the formation of a polysaccharide membrane is associated with a specific action of *A. xylinus*, which is connected with the hexose character of the carbohydrate in question, and evidently finding its most pronounced effect in the case of keto- rather than aldohexoses.

Experimental

Culture Media Employed

Yeast-water medium. Yeast-water*, 500 cc.; distilled water, 500 cc.; sucrose, 50 gm.; KH_2PO_4, 5.0 gm.; $MgSO_4$, 5.0 gm. This medium was tubed in 10-cc. portions. Subsequent to sterilization and immediately prior to use, absolute ethyl alcohol (0.5% by weight) was added to each tube.

Malt agar. Difco.

Wort gelatine. Hopped beer wort, 1,000 cc.; glucose, 50 gm.; gelatine, 150 gm.; pH adjusted to 5.0 ± 0.1.

Wort agar. Hopped beer wort, 1,000 cc.; glucose, 50 gm.; agar, 15 gm.; pH adjusted to 5.0 ± 0.1.

Additional media. Diagnostic media, *e.g.*, beef extract gelatine, beef extract agar, beef extract broth, nitrate broth, litmus milk and tryptophane broth, were prepared as outlined in the manual of the Society of American Bacteriologists (16).

All the above media were sterilized by autoclaving in the final containers for 20 min. at 15 lb. steam pressure.

Sugar broth. Solutions containing 2% of the carbon product under investigation, together with 0.5% of peptone and 0.1% of NaCl, were prepared by dissolving the constituents in distilled water. All the solutions were brought to pH 6.8 ± 0.1 by the addition of $N/10$ NaOH; 1.0 cc. of a 1.6% alcoholic solution of brom cresol purple per litre of solution was added as indicator. To avoid possible changes in the carbohydrates due to heat, the solutions were sterilized by filtering through Berkefeld "W" filters using suction (500 mm.). Portions (10 cc.) of each solution were then placed in sterile test tubes, and the sterility checked by incubating the solutions at 30° C. for three days.

**Compressed baker's yeast (½ kg.) is boiled for ½ hr. with distilled water (2 litres). The solids are removed by centrifuging, or by filtering the liquid while hot; the solid-free fluid constitutes the yeast-water employed throughout these investigations.*

Culture Employed

Several strains of acetic acid forming bacteria which gave a characteristic membrane ("Xylinumhaut") on suitable nutrient media were obtained through the kindness of Prof. R. O. Herzog, Director of the Kaiser Wilhelm Institute for Fibre Chemistry, Berlin-Dahlem, Germany. The comparative ability of these cultures to form the cellulosic membrane was ascertained by observing the relative amounts of this substance formed when these organisms were grown on yeast-water medium. The most active strain, judged by this technique, was purified by the plate method, employing malt agar as substrate, in order to ensure a pure strain for experimental work. After purification it coincided, in cultural and morphological respects, with the description of this species given by the Society of American Bacteriologists (16).

As this description is somewhat meagre, a summary of the cultural and biochemical characteristics is given here.

Cultural Characteristics

Beef extract broth: Small scattered zooglea, no clouding, extremely poor growth. *Beef extract agar:* Very poor growth, flat, transparent. *Beef extract gelatine stab:* Barely visible, filiform growth. *Potato:* Scanty, buff colored, filiform, moist. *Litmus milk:* Weak surface pellicle forms, litmus remains unchanged. *Wort agar:* Abundant growth, very adherent, dull, brown, raised; a distinct tough membrane forms on the water of condensation. *Wort gelatine:* Moderate growth, light brown, moist, raised.

Biochemical Characteristics. Nitrates are not reduced to nitrites; indol is not produced,

Production of Acid from Various Carbon Compounds

Duplicate 10-cc. portions of solutions of the carbon compounds being studied were inoculated with 0.1 cc. of a 1:100 dilution of the liquid of a week-old

TABLE I

NUMBER OF CC. OF $N/10$ SODIUM HYDROXIDE REQUIRED TO NEUTRALIZE THE ACID PRODUCED IN 10-CC. PORTIONS OF SOLUTIONS OF THE CARBON COMPOUNDS INDICATED

Carbon compound	Cc. $N/10$ NaOH required		Carbon compound	Cc. $N/10$ NaOH required	
	a	b		a	b
Starch	0.0	0.0	Arabinose	1.5	1.8
Dextrin	0.0	0.0	Xylose	1.7	1.5
Inulin	0.0	0.0	Glycol	1.2	1.3
Raffinose	0.3	0.3	Glycerol	1.6	1.6
Sucrose	0.6	0.4	Erythritol	0.7	0.8
Maltose	0.0	0.0	Mannitol	0.6	0.4
Lactose	0.0	0.0	*Ethyl alcohol	14.1	12.7
Glucose	4.8	4.9	†*Propyl alcohol	3.5	3.7
Galactose	0.7	0.6	†*Amyl alcohol	0.0	0.0
Fructose	0.3	0.3	Controls	0.0	0.0

**In order to avoid possible loss due to volatilization these alcohols were added to the nutrient medium immediately prior to inoculation.*

†It appeared that the bacterium would not develop in 2% solutions of these alcohols, and for this reason 0.5% solutions of amyl and propyl alcohols were employed.

yeast-water culture of the organism in distilled water. Subsequently all were incubated for 10 days at 30° C., and the total acid formed was then determined by titrating with $N/10$ NaOH until the original purple color of the indicator returned. The results are given in Table I.

This organism appears then to be a typical strain of *B. xylinum* (*A. xylinus*) according to Henneberg (11, p. 218), especially in regard to its fermentation reactions, for he states that: "Gesäuert wird Äthylalkohol (am meisten), Propylalkohol, Rohrzucker, etwas weniger Arabinose,—wenig Dextrose, Galaktose, Raffinose, Glyzerin, Glykol,—sehr wenig Lävulose und Mannit,—dagegen nicht Malzzucker, Milchzucker, Dextrin."

Optimum Conditions for the Production of the Cellulose Membrane Formed by *A. xylinus*

Brown's investigations (5) indicated that Pasteur's mineral solution (which contains 1000 cc. of distilled water; 0.1 gm. of ammonium tartrate;—or 1 gm. of ammonium carbonate; 1.0 gm. of ash from yeast, and 3% of glucose as a carbon source) could be employed with some success in the formation of the cellulosic membrane by *B. xylinum*. This author also observed that yeast water alone gave rise to a pronounced zoogleal formation. Herzog (12) states that the addition of ethyl alcohol accentuates the production of membranes by this type of organism. The researches of Henneberg (11, p. 190-240) indicate that acid phosphates are desirable constituents of synthetic media employed for the growth of acetic acid forming bacteria. Probably this is partly due to the fact that they buffer the medium at a pH desirable for growth.

It was therefore considered essential to obtain a source of nitrogen which, in combination with nutrient salts and ethyl alcohol, would yield a medium, in which the organism could not form zooglea, or give visible growth, but which, when a suitable carbon compound was added, would support the formation of an abundant amount of the cellulosic membrane, thus yielding a product formed practically entirely from the carbon derivative employed. Three types of nitrogen supply called for consideration: (a) a complex mixture of organic nitrogenous compounds, *e.g.*, peptone and yeast water; (b) a simple organic nitrogen compound; and (c) a solution containing inorganic nitrogen salts. The following experiments were conducted to establish which of the above types of nitrogen supply was most satisfactory. Brown (5) showed that yeast water alone gave rise to considerable zoogleal formation and therefore it appeared unnecessary to study this substance.

Experiment 1

A study was made of peptone as a nitrogen source. Solutions (100 cc.) containing 0.1% KH_2PO_4 and various concentrations of peptone (1.0; 0.5; 0.1; 0.05; and 0.01%) were prepared by dissolving the constituents in distilled water, placing each solution in a 250 cc. Erlenmeyer flask, and sterilizing at 15 lb. steam pressure for 20 min. Subsequently each solution was inoculated with 1-2 mm. loopful of a week-old yeast-water culture of *A. xylinus*, and the flasks

incubated at 30° C. After incubation for 10 days the flasks were examined and pronounced zoogleal formation was observed in all the solutions, particularly in that containing 1.0% of peptone.

These purely qualitative results indicated that peptone was an undesirable source of nitrogen, since it was desired to obtain a cellulosic material synthesized entirely from carbohydrate. Further experiments were therefore initiated to determine whether simple organic compounds of nitrogen or mineral nitrogen salts could be employed as nitrogen source.

Experiment 2

A comparison was made of the value of mineral salts and simple organic nitrogen compounds as nitrogen source in stimulating the formation of the cellulosic material by *A. xylinus*.

The solutions (60 cc.) specified in Table II were prepared by dissolving the constituents in distilled water.

TABLE II

COMPOSITION OF THE SOLUTIONS EMPLOYED IN EXPERIMENT 2

Solution No.	Percentage of compound in solution						
	KH_2PO_4	$(NH_4)_2SO_4$	NH_4Cl	KNO_3	NaCl	*Ethyl Alcohol	Glucose
1	2.5	1.0	1.0	1.0	0.5	0.5	5.0
2	1.25	0.5	0.5	0.5	0.25	0.5	5.0
3	0.5	0.2	0.2	0.2	0.1	0.5	5.0
4	2.5	1.0	1.0	1.0	0.5	0.5	0.0
5	1.25	0.5	0.5	0.5	0.25	0.5	0.0
6	0.5	0.2	0.2	0.2	0.1	0.5	0.0
		Asparagine					
7	0.5	1.0	...	...	...	0.5	5.0
8	0.5	0.5	...	...	...	0.5	5.0
9	0.5	0.05	...	...	...	0.5	5.0
10	0.5	0.005	...	...	...	0.5	5.0
11	0.5	1.0	...	...	...	0.5	0.0
12	0.5	0.5	...	...	...	0.5	0.0
13	0.5	0.05	...	...	...	0.5	0.0
14	0.5	0.005	...	...	...	0.5	0.0
		Urea					
15	0.5	1.0	...	...	...	0.5	5.0
16	0.5	0.5	...	...	...	0.5	5.0
17	0.5	0.1	...	...	...	0.5	5.0
18	0.5	0.01	...	...	...	0.5	5.0
19	0.5	1.0	...	...	...	0.5	0.0
20	0.5	0.5	...	...	...	0.5	0.0
21	0.5	0.1	...	...	...	0.5	0.0
22	0.5	0.01	...	...	...	0.5	0.0

**The ethyl alcohol was added in each case subsequent to sterilization, and immediately prior to inoculation of the medium.*

These solutions were sterilized by filtration through a Berkefeld "W" filter employing suction (500 mm.), and 50 cc. of each placed in sterile, cotton-plugged 100-cc. Erlenmeyer flasks of uniform shape. Any solution which

showed visible signs of contamination after 48 hours' incubation at 37.5° C. was replaced by a similar solution of proven sterility. Each solution was then inoculated with 0.01 cc. of the fluid portion of a 48-hour-old yeast-water culture of *A. xylinus*, and incubated at 30° C. The solutions were examined after 30 days' incubation and the following results obtained.

Solutions 1, 2, 4 and 5 showed no visible growth so that it may be assumed the concentration of nutrient salts in these media was too high to permit cell reproduction under the given experimental conditions. Solution 6 showed extremely slight zoogleal formation, while in solution 3 a well-defined polysaccharide membrane was formed. It was therefore concluded that solution 3 could be safely employed for the synthesis of the polysaccharide material from carbohydrate, since no sensible growth took place in a similar solution in the absence of carbohydrate.

Solutions 7, 8, 9 and 10 gave a well-defined polysaccharide membrane; while solution 9 appeared to yield a larger amount than the others; 11 and 14 showed no visible growth, while in 12 and 13 only extremely slight zoogleal formation occurred. Thus asparagine may be safely employed as nitrogen source.

Solutions 15, 19, 20, 21 and 22 gave no growth whatever. Solutions 16, 17 and 18 favored membrane formation, 17 giving better growth than either 16 or 18. It appeared therefore that these three solutions could be employed safely for the production of polysaccharide material from carbohydrates, since the controls gave no appreciable growth.

A comparative qualitative study of the amount of polysaccharide material formed in solutions 3, 9 and 17 immediately indicated that solution 9, which contained 0.05% of asparagine as nitrogen source, gave a far better growth than either of the other two. In fact, the membrane formation in the mineral salt and urea solutions in comparison with asparagine was exceedingly weak. Asparagine was therefore considered the most favorable nitrogen source for experimental work.

Experiment 3

The optimum concentrations of asparagine, potassium dihydrogen phosphate, sodium chloride, ethyl alcohol, and carbohydrate (glucose) favoring the formation of the polysaccharide material by *A. xylinus* were determined.

Solutions (60 cc.) of the compositions recorded in Table III were prepared similarly to the previous series (Experiment 2). After incubation for 14 days the relative amounts of polysaccharide material present were determined as follows: Filter papers (Whatman No. 30, 9 cm.) were washed thoroughly in boiling distilled water (three changes of 15 min. each) dried at 100° C. for 1 hr., then over $CaCl_2$ in a vacuum desiccator for 12 hr., and weighed. Each of the solutions was filtered in a Büchner funnel and full suction applied (approx. 60 cm.) until the polysaccharide product appeared free from visible traces of moisture. Three successive 100-cc. portions of hot distilled water (95° C.) were then poured on the filter, the polysaccharide material being freed from all visible traces of moisture between each addition by suction. The product was dried as above and weighed. The results are recorded in Table III.

TABLE III

COMPOSITION OF THE SOLUTIONS EMPLOYED IN EXPERIMENT 3 AND YIELDS OF POLYSACCHARIDE MATERIAL OBTAINED

No.	Asparagine %	KH_2PO_4 %	NaCl %	Glucose %	Ethyl* alcohol %	Weight of product,** gm.
1	0.5	0.5	0.1	2.0	0.5	0.014
2	0.25	0.5	0.1	2.0	0.5	0.026
3	0.1	0.5	0.1	2.0	0.5	0.021
4	0.05	0.5	0.1	2.0	0.5	0.019
5	0.025	0.5	0.1	2.0	0.5	0.014
6	0.01	0.5	0.1	2.0	0.5	0.012
7	0.05	1.0	0.1	2.0	0.5	0.018
8	0.05	0.5	0.1	2.0	0.5	0.016
9	0.05	0.1	0.1	2.0	0.5	0.014
10	0.05	0.05	0.1	2.0	0.5	0.013
11	0.05	0.01	0.1	2.0	0.5	0.012
12	0.05	0.5	1.0	2.0	0.5	0.011
13	0.05	0.5	0.5	2.0	0.5	0.020
14	0.05	0.5	0.1	2.0	0.5	0.036
15	0.05	0.5	0.05	2.0	0.5	0.027
16	0.05	0.5	0.01	2.0	0.5	0.018
17	0.05	0.5	0.001	2.0	0.5	0.018
18	0.05	0.5	0.000	2.0	0.5	0.017
19	0.05	0.5	0.1	10.0	0.5	0.034
20	0.05	0.5	0.1	5.0	0.5	0.027
21	0.05	0.5	0.1	2.0	0.5	0.024
22	0.05	0.5	0.1	1.0	0.5	0.020
23	0.05	0.5	0.1	2.0	2.0	0.026
24	0.05	0.5	0.1	2.0	1.0	0.067
25	0.05	0.5	0.1	2.0	0.5	0.077
26	0.05	0.5	0.1	2.0	0.25	0.058
27	0.05	0.5	0.1	2.0	0.00	0.002

*The ethyl alcohol was added after sterilization and immediately before inoculation.

**The weights in column 7 were in each instance calculated to the fourth place of decimals. However four control experiments indicated that, during the washing process each filter lost, on the average, 0.0011 gm.; accordingly the weights are expressed to the nearest third place of decimals.

The following conclusions may be drawn: (1) The optimum concentration of asparagine under such experimental conditions is 0.25%. Probably a concentration of 0.1% could be employed successfully for experimental work. (2) The highest concentration of KH_2PO_4 employed gave the highest yield of the polysaccharide product. However, the difference in the yield from solutions containing 1.0% and 0.5% of KH_2PO_4 was not very marked, and thus 0.5% of this salt should prove adequate for experimental purposes having regard to the isolation of a product relatively low in ash. (3) A concentration of 0.1% of NaCl gave the highest yield. (4) The highest concentration of glucose employed gave the highest yield, a result which naturally may not hold good in the case of other carbon compounds. For experimental purposes a concentration of 5% should be adequate. (5) The optimum concentration of ethyl alcohol appears to be 0.5%. The extremely low yield in the experiment

in which no ethyl alcohol was employed indicates that it is an important factor in producing pellicle formation. (6) These results when taken collectively indicate that a medium having the following composition should prove satisfactory for the production of the polysaccharide product by *A. xylinus* for experimental purposes:— asparagine, 0.1%; KH_2PO_4, 0.5%; NaCl, 0.1%; ethyl alcohol, 0.5% and carbon source, 5.0%.

In view of the fact that good yields of polysaccharide could be obtained by the use of the above medium it was considered unnecessary to study the possible increase in yield which might result from the addition of other inorganic salts (magnesium, calcium, etc.), especially since the addition of such salts might make it more difficult to obtain an ash-free product for chemical investigations.

Experiment 4

The time for maximum polysaccharide formation was determined as follows: Two solutions were prepared: (a) 10% glucose in distilled water, and (b) 1% KH_2PO_4; 0.2% NaCl; 0.2% asparagine dissolved in distilled water. Portions (100 cc.) of each solution were placed in 500-cc. Erlenmeyer flasks of uniform shape and these were sterilized in an autoclave. After cooling, portions of (a) and (b) were mixed under aseptic conditions. Absolute ethyl alcohol (0.5% by weight) was added to each flask, and the contents inoculated with 0.1 cc. of a 1:100 dilution of a week-old yeast-water culture of *A. xylinus* in distilled water*. The cultures were incubated at 30° C., and at intervals the amount of polysaccharide present was determined as described in Experiment 3.

The results are given in Table IV and may be accepted as evidence that the

TABLE IV

DETERMINATION OF TIME FOR MAXIMUM MEMBRANE FORMATION

Incubation period in days	3	4	5	6	9	10	12	14
Weight of polysaccharide in gm.	0.021	0.057	0.061	0.063	0.094	0.123	0.117	0.127

amount of polysaccharide formed does not increase appreciably after the tenth day of incubation. It was therefore decided to employ an incubation period of ten days for all experimental work.

A Study of Carbon Compounds Converted into a Cellulose-like Polysaccharide by a typical strain of *A. Xylinus*

In view of the results obtained by Brown (5) it appeared important to make a study of the carbon compounds assimilable by the organism under investigation with formation of a cellulose-like polysaccharide. The following experiments were carried out to determine the yields obtained using different media.

Experiment 5

The solution employed in Experiment 3 was used to furnish the requisite inorganic salts and nitrogen.

*The dilution was prepared by placing 0.1 cc. of the liquid from a week-old yeast-water culture of *A. xylinus* in 10 cc. of sterile distilled water, and mixing thoroughly.*

Solutions (110 cc.) containing 5 and 2.5% of the various carbon compounds under investigation were prepared by dissolving the derivatives in the following solution:—asparagine, 0.1%; KH_2PO_4, 0.5%; and NaCl, 0.1%, in distilled water, and adjusting the pH to 5.0 ± 0.1. These mixtures were sterilized by using a Berkefeld filter as before, and two 50-cc. portions of each filtrate were placed in sterile 100-cc. Florence flasks of uniform shape. The solutions were incubated for three days at 30° C. to check their sterility. Any solutions which showed visible contamination were replaced by similar solutions of proven sterility. Immediately prior to inoculation absolute ethyl alcohol (0.5% by weight) was added to each flask. Each solution was then inoculated with 0.1 cc. of a 1:100 dilution of *A. xylinus*, prepared as before. All solutions were incubated at 30° C. for ten days, care being taken not to disturb them, and the amount of polysaccharide material present in each determined as follows:

Sintered glass filters (Jena, 11 G.-2) were immersed for 15 min. in hot chromic acid solution, washed many times with distilled water, and dried at 100° C. for 2 hr., then finally over calcium chloride in a desiccator for 1 hr., and weighed. The solutions were filtered with suction (approx. 60 mm.), washed with three successive 50-cc. portions of hot water (95° C.), dried as above and weighed. The results are given in Table V together with those of Experiments 6 and 7 in order to facilitate comparison.

Experiment 6

In this experiment a medium was used containing peptone in addition to asparagine, together with certain inorganic salts not used in Experiment 5.

The procedure was identical with that of Experiment 5, except that a solution containing 0.1% asparagine, 0.1% peptone, 0.5% KH_2PO_4, 0.1% NaCl, 0.05% $MgSO_4$ and a trace of ferrous sulphate in distilled water, was employed as solvent for the various carbon derivatives. This solution was adjusted to pH 5.0 ± 0.1.

Experiment 7

A yeast-water medium was used as nutrient substrate. A medium having the following composition was prepared: yeast-water, 1000 cc.; asparagine, 0.2%; KH_2PO_4, 1.0%; NaCl, 0.2%; $MgSO_4$, 0.1%; and ferric sulphate, trace. The pH was adjusted to 5.0 ± 0.1. Portions (25 cc.) of this medium were measured into 100-cc. Florence flasks of uniform shape, and the resulting solutions sterilized in the autoclave.

Solutions (10%) of the various carbon compounds under investigation were prepared by dissolving them in distilled water, heating where necessary (but not over 100° C.). The starch solutions were sterilized by autoclaving and the others by filtration. Portions (25 cc.) of the resulting solutions were then placed in each of two flasks containing 25 cc. of the yeast-water medium. Sterility was checked as before. Absolute ethyl alcohol (0.5% by weight) was then added to each solution immediately before inoculation. After incubation the amount of cellulosic polysaccharide was determined exactly as outlined in Experiment 5.

TABLE V

YIELD OF SYNTHETIC POLYSACCHARIDE FROM CARBON COMPOUNDS

Carbon source, (volume of solutions, 50 cc.)	Weight of synthetic polysaccharide, gm.					
	Experiment 5 Standard nutrient solution		Experiment 6 Standard nutrient solution + peptone + $FeSO_4$ + $MgSO_4$		Experiment 7 Yeast-water nutrient medium	
	Duplicates	Average	Duplicates	Average	Duplicates	Average
Controls (No added carbon compound)	0.0000 0.0000	0.0000	0.0007 0.0005	0.0006	0.0645 0.0681	0.0663
Soluble starch, 5%	0.0018 0.0024	0.0021	0.0000 0.0000	0.0000	0.0887 0.0793	0.0840
Soluble starch, 2.5%	0.0005 0.0003	0.0004	0.0000 0.0000	0.0000	0.0446 0.0411	0.0429
Inulin, 5%	0.0245 0.0238	0.0242	0.0258 0.0251	0.0255	0.0822 0.0778	0.0800
Inulin, 2.5%	0.0208 0.0217	0.0213	0.0185 0.0196	0.0191	0.0546 0.0492	0.0519
Levan,* 5%	0.0075 0.0085	0.0080	0.0080 0.0062	0.0071	0.0590 0.0511	0.0551
Levan, 2.5%	0.0070 0.0063	0.0067	0.0026 0.0032	0.0029	0.0312 0.0291	0.0302
Dextrin, 5%	0.0013 0.0009	0.0011	0.0019 0.0025	0.0022	0.0410 0.0387	0.0399
Dextrin, 2.5%	0.0000 0.0000	0.0000	0.0009 0.0013	0.0011	0.0171 0.0195	0.0183
Raffinose, 5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0305 0.0287	0.0296
Raffinose, 2.5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0309 0.0318	0.0314
Sucrose, 5%	0.0160 0.0186	0.0173	0.0142 0.0121	0.0132	0.0794 0.0837	0.0816
Sucrose, 2.5%	0.0112 0.0091	0.0102	0.0145 0.0166	0.0156	0.0555 0.0495	0.0525
Lactose, 5%	0.0150 0.0113	0.0132	0.0007 0.0005	0.0006	0.0583 0.0560	0.0572
Lactose, 2.5%	0.0058 0.0044	0.0051	0.0000 0.0000	0.0000	0.0491 0.0469	0.0480
Maltose, 5%	0.0023 0.0018	0.0021	0.0381 0.0402	0.0392	0.0805 0.0762	0.0784
Maltose, 2.5%	0.0012 0.0013	0.0013	0.0111 0.0088	0.0100	0.0443 0.0498	0.0471
Glucose, 5%	0.0486 0.0512	0.0499	0.0911 0.0851	0.0881	0.1511 0.1598	0.1555

**The levan was prepared according to the method described in a previous communication* (*10*).

TABLE V—*Continued*

Carbon source, (volume of solutions, 50 cc.)	Weight of synthetic polysaccharide, gm. Experiment 5 Standard nutrient solution: Duplicates	Experiment 5: Average	Experiment 6 Standard nutrient solution + peptone + $FeSO_4$ + $MgSO_4$: Duplicates	Experiment 6: Average	Experiment 7 Yeast-water nutrient medium: Duplicates	Experiment 7: Average
Glucose, 2.5%	0.0311 0.0342	0.0327	0.0691 0.0635	0.0663	0.1494 0.1458	0.1476
Fructose, 5%	0.2646 0.2416	0.2531	0.2478 0.2567	0.2523	0.3857 0.3918	0.3888
Fructose, 2.5%	0.1040 0.1158	0.1099	0.1595 0.1600	0.1598	0.1733 0.1688	0.1711
Mannose, 5%	0.0028 0.0025	0.0027	0.0004 0.0002	0.0003	0.0697 0.0661	0.0679
Mannose, 2.5%	0.0018 0.0015	0.0017	0.0000 0.0000	0.0000	0.0257 0.0289	0.0273
Galactose, 5%	0.0340 0.0301	0.0326	0.0198 0.0211	0.0205	0.0597 0.0635	0.0616
Galactose, 2.5%	0.0310 0.0288	0.0299	0.0212 0.0238	0.0225	0.0475 0.0501	0.0488
Xylose, 5%	0.0000 0.0000	0.0000	0.0003 0.0006	0.0005	0.0424 0.0399	0.0412
Xylose, 2.5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0316 0.0288	0.0302
Arabinose, 5%	0.0000 0.0000	0.0000	0.0007 0.0013	0.0010	0.0302 0.0279	0.0291
Arabinose, 2.5%	0.0000 0.0000	0.0000	0.0005 0.0008	0.0007	0.0239 0.0208	0.0224
Rhamnose, 5%	0.0012 0.0015	0.0014	0.0007 0.0009	0.0008	0.0607 0.0618	0.0613
Rhamnose, 2.5%	0.0007 0.0007	0.0007	0.0003 0.0001	0.0002	0.0406 0.0377	0.0392
α-Methyl glucoside, 5%	0.0000 0.0000	0.0000	0.0009 0.0011	0.0010	0.0243 0.0265	0.0254
α-Methyl glucoside, 2.5%	0.0000 0.0000	0.0000	0.0006 0.0003	0.0005	0.0182 0.0218	0.0200
Glycol, 5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0210 0.0220	0.0215
Glycol, 2.5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0077 0.0072	0.0075
Glycerol, 5%	0.0801 0.0793	0.0797	0.0795 0.0851	0.0823	0.2474 0.2586	0.2530
Glycerol, 2.5%	0.0533 0.0418	0.0476	0.1019 0.1011	0.1015	0.1017 0.0998	0.1008

TABLE V—*Continued*

Carbon source, (volume of solutions, 50 cc.)	Weight of synthetic polysaccharide, gm.					
	Experiment 5 Standard nutrient solution		Experiment 6 Standard nutrient solution + peptone + $FeSO_4$ + $MgSO_4$		Experiment 7 Yeast-water nutrient medium	
	Duplicates	Average	Duplicates	Average	Duplicates	Average
Erythritol, 5%	0.0000 0.0000	0.0000	0.0006 0.0011	0.0009	0.0425 0.0429	0.0427
Erythritol, 2.5%	0.0000 0.0000	0.0000	0.0003 0.0003	0.0003	0.0313 0.0289	0.0301
Mannitol, 5%	0.0899 0.0884	0.0892	0.1420 0.1460	0.1440	0.4021 0.3897	0.3959
Mannitol, 2.5%	0.0700 0.0773	0.0737	0.1561 0.1534	0.1548	0.1806 0.1846	0.1826
α-Methyl glycerol, 5%	0.0008 0.0010	0.0009	0.0011 0.0012	0.0012	0.0035 0.0047	0.0041
α-Methyl glycerol, 2.5%	0.0000 0.0000	0.0000	0.0003 0.0005	0.0004	0.0028 0.0031	0.0030
Glycerol chlorhydrin, 5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0020 0.0027	0.0024
Glycerol chlorhydrin, 2.5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000
l-Glucosan, 5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0535 0.0503	0.0519
l-Glucosan, 2.5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0223 0.0209	0.0216
Tetra-ethylene glycol, 5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0332 0.0401	0.0367
Tetra-ethylene glycol, 2.5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0139 0.0178	0.0159
Hexa-ethylene glycol, 5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0297 0.0318	0.0308
Hexa ethylene glycol, 2.5%	0.0000 0.0000	0.0000	0.0000 0.0000	0.0000	0.0098 0.0112	0.0105
Peptone, 1%	....	......	0.0075 0.0070	0.0073	....	......
Peptone, 0.5%	0.0016 0.0019	0.0018	0.0080 0.0093	0.0087	....	......
Peptone, 0.1%	0.0005 0.0007	0.0006	....	......	....	......

When the weight of the synthetic material obtained in the controls of each experiment is compared with that obtained after the addition of the various carbon compounds to similar solutions the results of Experiments 5, 6 and 7 may be interpreted as follows:

(1) The carbon compounds most suitable for synthetic polysaccharide formation are essentially the same for each of the three nutrient solutions employed. The fact that certain carbon derivatives, which yield appreciable amounts of synthetic polysaccharide when used with the nutrient solution indicated in Experiment 1, do not give an increased yield in the peptone medium (Experiment 6), may be attributed to the fact that they are more readily transformed into other soluble products in the richer nutrient solutions.

(2) The carbohydrates giving the highest yields of synthetic polysaccharide are the hexoses. Fructose appears to be by far the most reactive of the hexoses, and this activity doubtless stands in close relationship to the very small amount of acid formation. On the other hand glucose, which undergoes oxidation to gluconic acid (17, p. 164), gives a much lower yield than fructose. The reason that galactose and mannose give far smaller yields of polysaccharide than either fructose or glucose cannot as yet be explained, but this is related presumably to the structural differences of the molecules in question.

(3) Neither of the pentoses examined, arabinose and xylose, undergoes assimilation by *A. xylinus* with polysaccharide formation.

(4) Since *A. xylinus* has been shown to oxidize erythritol at the β-carbon atom with formation of *d*-erythrulose (17, p. 166) it would seem that tetroses are not suitable sources for the formation of polysaccharide products.

(5) The experimental results show that glycerol gives a very high yield of synthetic polysaccharide. *A. xylinus* is known to oxidize glycerol at the β-carbon atom with formation of dihydroxy acetone (17, p. 166) and hence it seems likely that two molecules of the dihydroxy acetone may polymerize to form fructose, the latter being then converted into the polysaccharide. Mannitol, which is known to be oxidized at the β-carbon atom to form fructose (17, p. 164), was also found to give a high yield of polysaccharide.

(6) The disaccharides, lactose, sucrose and maltose, all give rise to polysaccharide formation, but the yields are lower than from fructose and glucose. It is probable that a preliminary hydrolysis of the disaccharide molecule must take place before polymerization can proceed. The only trisaccharide employed, raffinose, was found to be inactive.

(7) The fructofuranose polysaccharides, levan and inulin, give quite low but yet appreciable yields of a synthetic polysaccharide, while those derived from glucopyranose units, starch and dextrin, give an extremely low yield. Probably, as in the case of the disaccharides, a preliminary hydrolysis is necessary, although this remains to be proved.

(8) Ethylene glycol, which is not oxidized to a ketose by the type of organism under investigation (17, p. 166) does not give rise to polysaccharide formation, and this is also true of the two polyglycols investigated, tetra- and hexaethylene glycols.

(9) The presence of a single methyl group appears to prevent polysaccharide formation completely, as indicated in the case of α-methyl glycerol and α-methyl glucoside respectively. The methyl pentose, rhamnose, is also quite inactive. The introduction of a chlorine atom, as in glycerol chlorhydrin, greatly retards growth and polysaccharide formation.

(10) It is of interest that the addition of peptone considerably increases the amount of synthetic material formed in the absence of other carbon compound, although the reason for this remains unknown.

As a result of the numerous experiments outlined above the following method was found to give the most satisfactory product and yield.

Preparation of a Cellulose-like Polysaccharide for Chemical Investigations

The following solutions are prepared: – Solution (a): 5-10% of the carbon compound in tap water. Solution (b): 1% KH_2PO_4; 0.2% NaCl and 0.2% asparagine dissolved in tap water.

These solutions, in 500-cc. portions, are sterilized in one-litre Erlenmeyer flasks in an autoclave for 20 min. at 15 lb. steam pressure*. The solutions are then cooled, and (a) and (b) mixed carefully, observing aseptic conditions. Absolute ethyl alcohol (0.5% by wt.) is added to each flask, and each solution inoculated with 0.1 cc. of a 1:100 dilution of a week-old culture of *A. xylinus* in sterile distilled water. After incubating for three days at 30° C. they are poured carefully into large sterile glass moisture chambers (7 by 22 cm.). The solutions are re-incubated in these containers for an additional seven days at 30° C., care being taken not to disturb the formation of the surface membranes. The cellulosic membranes are then removed and prepared for chemical investigation (13). The yield is relatively low, *e.g.*, 35 litres of culture medium containing 1750 gm. of glucose, yielded only 72 gm. of the pure dried membrane, *i.e.*, approximately 4.1% of the glucose used.

One-litre portions of control solutions which contained the mineral salts, asparagine and ethyl alcohol as in the usual medium, but no added carbon compound, showed after 10 days' incubation at 30° C. absolutely no visible signs of growth.

The synthetic polysaccharide formed by the action of *A. xylinum* on glucose is under investigation in these laboratories by Mr. J. Barsha, who has been able to show that it is converted quantitatively into the characteristic mixture of α- and β-methyl glucosides by hydrolysis with methyl alcohol containing hydrochloric acid. The triacetate and trimethyl derivatives have been prepared and are under investigation. As noted in a previous communication (10) the structure of the polysaccharide membranes formed by *A. xylinus* on a number of other carbon compounds is being investigated.

**In cases where the carbon derivative employed is relatively unstable on heating, the sterilization of the solution is performed by filtering through a Berkefeld "W" filter, employing suction (500 mm.), placing 500-cc. portions in one-litre Erlenmeyer flasks, and incubating three days at 30° C. in order to check the sterility.*

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STUDIES IN THE VARIABILITY OF TUBERCLE BACILLI
I. A RAPID-GROWING BOVINE TYPE[1]

By Guilford B. Reed[2] and Christine E. Rice[3]

Abstract

Variability studies have been carried out on a considerable series of cultures of tubercle bacilli and related species of acid-fast bacteria. The present paper deals with a rapid-growing strain of the bovine tubercle bacillus. The results are in conformity with claims recently made by Petroff that certain types of tubercle bacilli undergo dissociation.

It is shown that this avirulent, rapid-growing strain consists of R types which appear to be stable in acid fluid media and more or less stable on solid media. Rapid transfers through strongly alkaline fluids or growth in large volumes of similar media result in appreciable dissociation into S types. The S types were unstable, particularly in acid fluid media, and readily reverted to the R form.

Introduction

The following paper is the first of a series which will be published from this laboratory in the near future, dealing with variability or dissociation in several species of tubercle and other acid-fast bacteria. The work was prompted by the now voluminous studies on B.C.G. of Calmette and Guerin. It was originally undertaken in order to obtain, if possible, more information concerning the changes which presumably went on in this culture in the course of its alteration, in Calmette's hands, from a state of high virulence to one of low virulence and at the same time to re-examine the fixity of this low degree of virulence.

It seemed at once that these changes were associated with the now extensively studied and firmly established phenomena of dissociation as they appear in many other species of bacteria. Since this work was undertaken Petroff and associates, (12, 13, 14) have clearly shown that several species of tubercle bacilli may be dissociated into two or more definite types, which differ from each other in colony form and in virulence for laboratory animals. The supposed dissociated cultures of B.C.G. sent to Europe by Petroff were examined by Kraus (10) who found one type to be virulent and one avirulent, by Gerlack (5) who found both to be avirulent, and by Lange (11), who found one type to be virulent but considers it to be a human tubercle bacillus and therefore presumably to result from contamination rather than dissociation of the bovine B.C.G. Several observers working with other cultures of B.C.G. have confirmed Petroff's results in so far as colony form is concerned. Begbie (1) described two colonies of B.C.G. Kraus (10), Tzekhnovitzer (18), Gerlach (5) and Piasecka-Zeyland (15) also observed two colony types in cultures of B.C.G. but did not find that difference in colony form was associated with difference

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Contribution from the Department of Bacteriology, Queen's University, Kingston. This work has been carried on with the co-operation and financial assistance of the Associate Committee on Tuberculosis of the National Research Council.

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in virulence. Kahn and Schwartzkopf have recently confirmed Petroff's results with avian tubercle bacilli and at the same time enlarged the field by demonstrating differences in the electrophoretic potential of organisms from the two colony types. It is of the greatest importance, not only to the B.C.G. situation, but to the whole problem of tubercle infection to determine the validity of dissociation of the tubercle bacilli.

This first paper deals with an atypical rapid-growing tubercle bacillus. Later papers will be concerned with more typical strains of tubercle bacilli, *Mycob. leprae*, *Mycob. phlei* and in less detail with several similar species. It also became evident that for adequate conclusions concerning variability other criteria than colony form and virulence were essential. Accordingly studies were undertaken, parallel with observations on alterations in colony structure and in virulence, on acid agglutination and isoelectric points of suspensions, on electrophoretic potentials, on oxygen consumption and optimum oxidation-reduction potentials, on the lipoid content, and perhaps most important of all, on the antigenic structure of variant types. These considerations will form the subject of subsequent papers.

Mycobacterium Tuberculosis Bovis No. 599

In 1925 there was received from the American Type culture collection, a rapid-growing culture of tubercle bacilli labelled *Mycob. tuberculosis Bovis* No. 599. Dr. Weaver, the curator, has kindly supplied the information that this culture was isolated in 1921 by workers at the University of Buffalo from tubercles in the lung of a cow. A culture was sent from the collection later the same year to Dr. Lynch of the Laurel Sanitarium, Sheldon, Conn., who reported five years later that the culture was non-pathogenic. It would seem probable that the organism was related to the lesion in the cow from which it was isolated and was therefore of some pathogenicity. If so, it had apparently lost this characteristic during the period of cultivation until tested by Dr. Lynch. It is of course possible that the organism under examination is not the one isolated from the bovine tuberculous material.

When received in this laboratory, it produced a rapid growth on all the media on which it was grown. Growth was generally visible in three to five days and at a maximum in ten to fifteen days. Where heavy inoculation was made on such media as Dorsett's egg, the growth resembled an ordinary bovine type though rather more prolific, more deeply pigmented where available iron was present in the medium, and the surface of the growth rather smoother, approaching in this respect the appearance of the avian type.

Colony Forms of the Original Culture, R Colonies

The culture was maintained for a period of approximately three years after it was received from Chicago, on Petroff's gentian violet-egg medium with transfers every two to three months. With the beginning of this study the culture was plated also on Petroff's medium, in such a way that well-separated individual colonies developed, not more than 200-300 to a Petri plate. This

was effected by grinding the inoculum in buffered saline, filtering through paper as described by Petroff (14) or by centrifuging to remove clumps and inoculating with the water-clear filtrate or centrifugate.

The colonies presented a variety of forms, but in this series the chief differences appeared to be the result of a difference in the age of the culture. Very young colonies just visible on the surface of the medium generally exhibited hemispherical mounds with a regular outline and a smooth waxy surface. These enlarged without any change in form until they reached a diameter of two to three millimetres (Plate I, Fig. 1). In some cultures there was no further change. Generally as the colony reached this diameter it became more or less umbilicate and one or two furrows appeared across the surface of the colony (Plate I, Fig. 2 and 3). As growth proceeded more furrows frequently developed, deepening gradually until the colony appeared partly divided into segments, (Plate I, Fig. 4). Still further growth generally resulted in extensive folding of the surface of the colony, (Plate I, Fig. 5). In others this separation into segments was less conspicuous or absent; the regular hemispherical form being followed by the formation of a central depression which became more and more conspicuous as a result of growth of the margin of the colony throwing it at first into a ring-like structure and then, if conditions remained favorable for growth, into an irregular folded surface (Plate II, Fig. 6). In the initial study a great many of these different colonies were isolated and plated on gentian violet egg medium; none bred true, suggesting, as did the progress of a single colony, that they represented simply growth phase of a single type.

It will be shown later that other strains of tubercle bacilli and other species of acid-fast bacteria produce colonies closely resembling these just described both in form and in development.

In conformity with the term used by Petroff (12) in his work on the dissociation of tubercle bacilli this colony is styled R. It will be shown in later papers that this colony form is generally associated with low virulence and with lack of antigenic complexity, and certain physico-chemical characteristics which are approximately constant throughout the acid-fast group. From the early work of Arkwright, DeKruif, Weil and Felix, Griffith and many others, these characteristics have been associated with roughness. In this group the terms rough and smooth cannot be used as specific description of the colony structure. The terms R and S are retained merely as convenient symbols to designate two distinct types within the well-defined limits of the species.

S Colonies

During the course of some ten culture generations of the original type on Petroff's gentian violet medium, many thousand colonies were closely observed. The great majority exhibited the typical R form as just described. A few however appeared flatter, more granular, and tended to spread out on the surface of the medium. Many of these were fished and re-planted on similar media. The progeny consisted in part of characteristic R forms and in part of flat granular forms showing various degrees of departure from the R type.

Such a mixture of types is shown in Plate II, Fig. 7, in the low magnification. Plate II, Fig. 8, 9 and Plate III, Fig. 10 show several intermediate colonies with S margins and R centres at the same magnification as the pure R types shown in earlier figures. By successive isolation of the most extreme of these flat, spreading, granular colonies, strains were eventually established which bred approximately true to type.

When mature, this new colony presents on the gentian violet egg medium, the form of a very irregular, much flattened cone with a fine granular surface. The colony slopes from its raised central area to a thin, very irregular outline at the surface of the medium (Plate III, Fig. 11, 12, 13 and 14). Old colonies frequently exhibit some folding. When these colonies are lifted with a loop, they tend to come away from the medium in one piece, and on grinding at first break into the individual granules composing their structure in contrast to the behavior of the more slimy R colony.

It will be shown in later papers that other tubercle bacilli and other species of acid-fast organisms produce S colonies similar to these just described.

Dissociation in Fluid Media

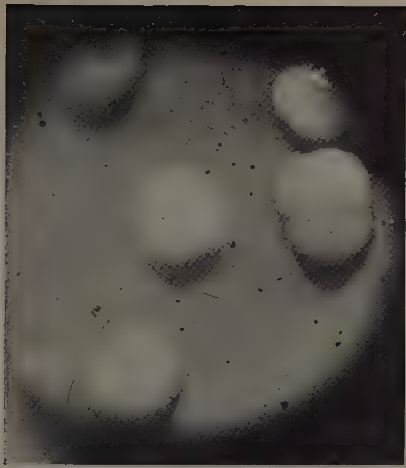
It has generally been observed that variation in bacteria takes place more readily when they are grown in fluids than on solid media. Soule (17) regards dissociation as a growth phenomenon and considers that in fluid media conditions permit of a longer period of logarithmic growth than on solids where the accumulation of metabolic products soon inhibits rapid growth. At the same time, it is reported by several workers (3, 7, 9, 21) that the best means of maintaining vigorous cultures of the S form of a number of species is rapid transfer in liquid media. Soule (17), on the other hand, found no change in R strains of *B. subtilis* consequent upon rapid transfer in broth.

TABLE I

THE EFFECT OF RAPID TRANSFER THROUGH INFUSION BROTH ON THE RATE OF R TO S DISSOCIATION

Broth generation	Colony types from			
	1st colony	2nd colony	3rd colony	4th colony
1	100R	100R	100R	100R
2	100R	100R	100R	100R
3	100R	100R	99R, 1S	100R
4	100R	100R	100R	99R, 1S
5	94R, 6S	100R	100R	91R, 5S, 4RS
6	—	100R	100R	98R, 2S
7	—	100R	63R, 17S, 20RS	94R, 2S, 4RS
8	—	100R	97R, 3S	—

NOTE:—*In this and subsequent tables showing the proportionate occurrence of colony forms, the figures are only approximations, since many colonies cannot be classified without extended subculturing. As shown in the text, however, enough subculturing has been done to indicate that the figures are not far from correct.*



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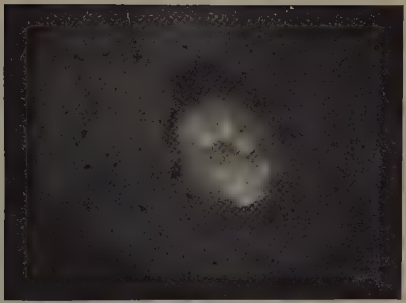

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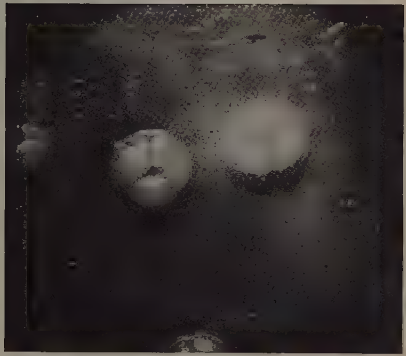

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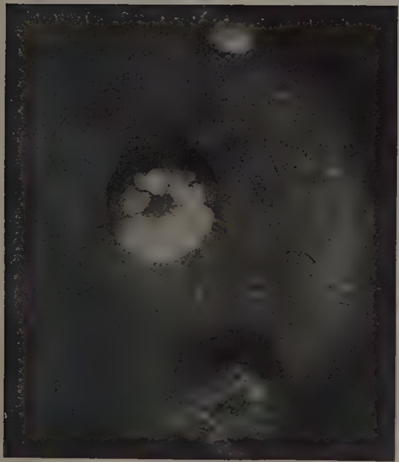

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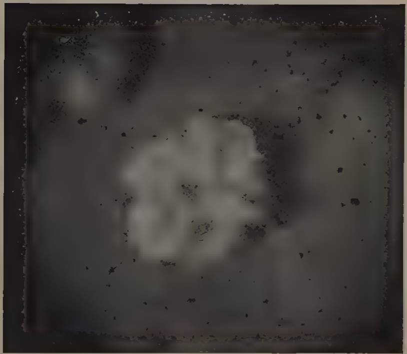
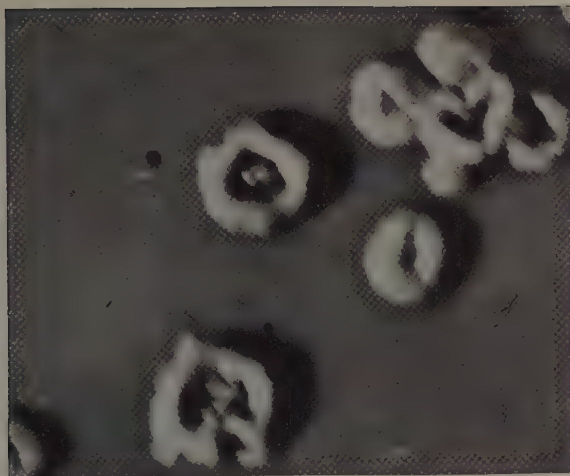


5

Photomicrographs of R colonies growing on gentian violet-egg media. Magnification 10 times.
FIG. 1. *Young colonies showing regular mounds.* FIG. 2. *Slightly older umbilicate colony.* FIG. 3 AND 4. *Colonies showing divisions into segments.* FIG. 5. *Fully matured colony with a much-folded surface growth.*



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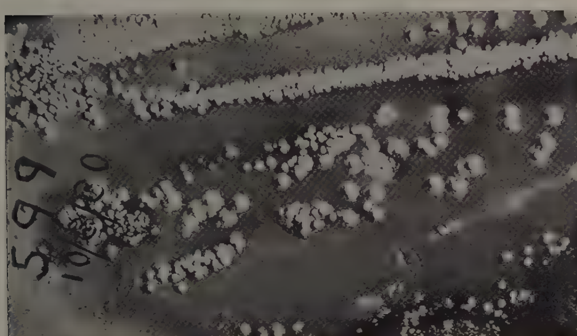

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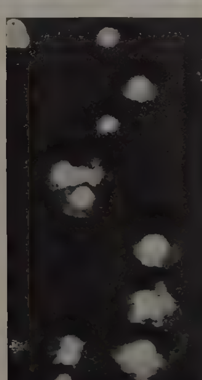

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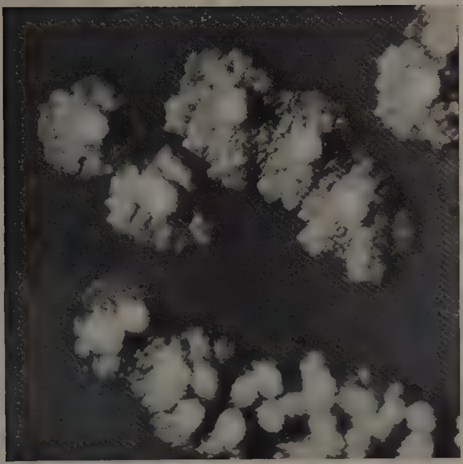
Photomicrographs of R and intermediate colonies growing on gentian violet-egg media.

FIG. 6. *Ring-shaped colonies, magnified 10 times.* FIG. 7. *A group of colonies many of which show intermediate forms, S margins and R centres, magnified 2 times.* FIG. 8 AND 9. *Similar intermediate colonies magnified 10 times.*



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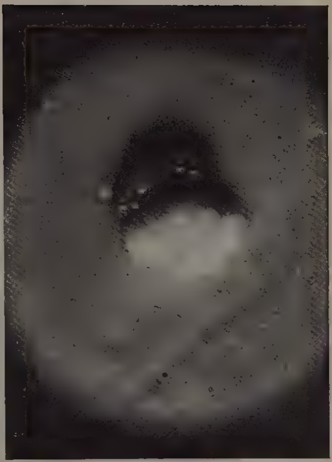

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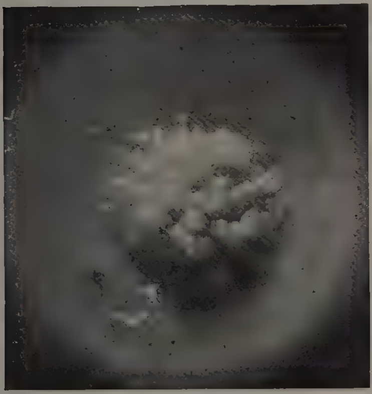

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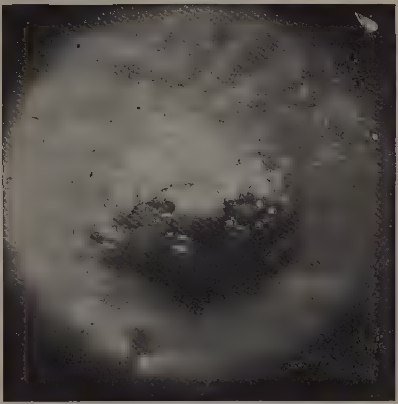

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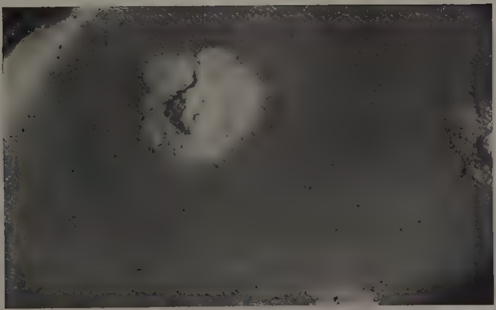

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Intermediate and S colonies growing on gentian violet-egg media.

FIG. 10. *Intermediate colonies showing definite S margins and indefinite R centres, magnification 10 times.* FIG. 11, 12, 13 AND 14. *S colonies, magnification 10 times.*

Several experiments have been made in which R colonies were planted in broth and transferred as rapidly as possible from broth to broth. In one series, four characteristic R colonies were selected from a series which had for several generations produced only R and transferred to tubes of beef-infusion glycerol broth, pH 7.8. After five days' growth, transfers were made to fresh broth and this repeated through some eight to nine generations. Plates were made at the end of each broth generation on gentian violet egg. The results are indicated in Table I.

Several of the S colonies which appeared on these plates were isolated. Although some bred true, the majority produced some S, some intermediate and generally some R types. After repeated subculturing of the most definite S types, more or less stable true-breeding strains were established.

It seems evident from the results shown in Table I that repeated transferring in broth tends to promote dissociation, but that there is a great individual difference in the behavior of colonies since of four similar colonies selected at the same time, one produced no such dissociation. This experiment was repeated several times during a period of two years. Similar results were obtained but the frequency of appearance of S types varied enormously.

Several observers (3, 7, 19) have found that large volumes of fluid media promote variation. Soule (17) in extending his thesis, previously referred to, that dissociation is a growth phenomenon, considers that increasing the volume of fluid increases the growth period and finds that the type which predominates at the start shows the greatest increase as if the reaction followed the mass law. In order to test the influence of large amounts of fluid, eight characteristic R colonies were selected from a series which had been subcultured for six generations from single R colonies and had produced only R colonies. These eight colonies were transferred to eight 500-cc. Erlenmeyer flasks containing 200 cc. of beef-extract glycerol broth buffered with phosphate and adjusted to pH 8.0. These were plated on Petroff's gentian violet egg

TABLE II

THE EFFECT OF LONG CULTIVATION ON LARGE VOLUMES OF BROTH (200 CC.) ON THE RATE OF R TO S DISSOCIATION

Colony No.	Period of growth in broth		
	5 days	8 days	23 days
	R to S	R to S	R to S
1	1800 – 1	600 – 0	1000 – 2
2	2800 – 0	600 – 0	700 – 9
3	3000 – 17	300 – 0	300 – 0
4	500 – 1	300 – 1	3500 – 30
5	—	600 – 1	1500 – 13
6	200 – 0	600 – 0	400 – 1
7	600 – 0	200 – 0	100 – 5
8	—	500 – 0	200 – 3
Total	8900 – 19	3700 – 2	7700 – 64
	450 – 1	1850 – 1	161 – 1

medium after five, eight and twenty-three days' growth. The results shown in Table II indicate a considerable amount of R to S dissociation though not more than in the case of rapid transfers in small volume.

Since, as shown in Table II, the greatest number of S forms appeared in the older culture, a second series of four R colonies was selected from a different series of plates and transferred to 250-cc. flasks containing 100-cc. amounts of beef-extract glycerol broth, pH 7.8. Plates were made at intervals of 7 to 40 days. The results are shown in Table III. Some dissociation had apparently

TABLE III

THE EFFECT OF LONG CULTIVATION ON LARGE VOLUMES OF BROTH (100 CC.) ON THE RATE OF R TO S DISSOCIATION

Colony No.	Period of growth in broth					
	7 days	10 days	15 days	20 days	30 days	40 days
1	90R, 6S, 4RS	90R, 5S, 5RS	80R, 15S, 5RS	80R, 19S	97R, 2S, 1RS	99R, 1S
2	100R	100R	63R, 33S, 4RS	77R, 21S, 2RS	75R, 15S, 10RS	94R, 3S, 3RS
3	100R	100R	98R, 2S	100R	97R, 3S	—
4	100R	100R	100R	100R	100R	100R

occurred when the first plates were made at the seventh day, but the maximum amount appeared after 15 to 20 days' growth in the broth and this period, it may be noted, is near or just beyond the end of the logarithmic growth period. This has also been observed in several subsequent experiments and apparently confirms Soule's (17) contention that dissociation is a growth phenomenon. As in previously recorded experiments apparently similar R colonies used as initial inoculum exhibited varying degrees of dissociation.

Influence of Concentration of Nutrients

Glycerol is generally included in media for tubercle bacilli though it is known to be somewhat toxic, at least to freshly isolated bovine strains. A series of beef-infusion broth flasks was made up containing 0, 2.5, 5 and 10% of glycerol, adjusted to pH 7.8 and all inoculated with an R culture which had shown some tendency to dissociate. In the absence of glycerol, growth was scanty; in the presence of 10% glycerol growth was less than normal and the pellicle slimy. At weekly intervals up to four weeks plates were made on gentian violet egg from the series of broth cultures. The results are shown in Table IV. It is apparent that the cultures without glycerol, even though the growth was very scanty, gave the higher yield of S types. It should be noted however that this flask remained strongly alkaline whereas the glycerol cultures showed a slight change in pH; the results may therefore be largely due to the acidity.

TABLE IV

THE INFLUENCE OF GLYCEROL CONCENTRATION ON R AND S DISSOCIATION

Concentration of glycerol, %	Period of growth in broth				Final pH
	7 days	14 days	21 days	28 days	
0	75R, 5S, 20RS	95R, 3S, 2RS	90R, 8S, 2RS	50R, 5S, 45RS	8.0
2.5	100R	92R, 8S	88R, 2S	100R	6.7
5.0	99R, 1S	84R, 7S, 9RS	96R, 2S, 2RS	99R, 1S	7.4
10.0	99R, 3S, 1RS	100R	100R	—	7.5

Altering the concentration of peptone and altering the total concentration of nutrients reduced the growth rate and to a slight extent retarded the R to S transformation.

Effects of Immune Serum

Numerous workers have observed an accelerated rate of dissociation on growing the bacteria in a homologous antiserum. Following this procedure Petroff (12) grew B.C.G. (which is clearly an R type of tubercle bacilli) in 10% anti-B.C.G. rabbit serum for seven to eight generations and on plating on gentian violet egg medium, he claims to have obtained definite dissociation to S. The writers accordingly immunized several rabbits with the R type of Bovine No. 599 using both heat-killed emulsions and a Dryer preparation of similar forms. On adding this immune sera in 10% amounts to extract broth no conspicuous increase in rate of change from R to S was observed over that occurring in the broth without serum.

Influence of Iron on the R to S Dissociation

In an earlier paper (16), we reviewed the work which has been done on the influence of iron on the growth of tubercle bacilli and found some fresh evidence of its growth-promoting effects. It was therefore estimated that it might have some influence on variability. Several experiments were carried out with rapid transfers of R types in broth containing the amount of iron previously determined for best promotion of growth. A slightly higher proportion of S types developed than in cultures in broth identical except for the absence of iron. The difference, however, was not greater than the difference in growth rate in the iron and the iron-free media.

Stability of R Type

In the previous sections it has been shown that the dissociation of R to S does not take place with equal frequency in all R cultures. This was most apparent in experiments where several similar R colonies were fished from the same plate and subjected to the same cultural conditions as summarized in Tables I, II and III. Some exhibited conspicuous variation, some a lesser rate of dissociation and some remained stable or bred true to the parent R type. These experiments recorded in the former section were carried out

during the winter of 1927 and a few in 1928. During the winter of 1930 an attempt was made to extend certain of the observations. The stock cultures of the R type, which had been maintained for the intervening two years on tubes of Petroff's gentian violet egg medium by transferring at one- to two-month intervals, exhibited complete stability.

Stability of the S Type

In beef-extract glycerol broth buffered with phosphate at pH 7.8 to 8.0 the S types have remained stable for a considerable period. In one instance four S colonies were transferred to a corresponding number of flasks of the extract broth, pH 7.8, and carried through three transfers in the broth at twelve-day intervals. Plates made from the last broth series still produced all S colonies. In similar media adjusted to a reaction of pH 6.5 to 6.8 the S types have proved to be highly unstable, after three to four culture generations plates have always shown from 10 to 15% R colonies.

On most solid media, the S type has proved to be highly unstable. Successive culture on Petroff's gentian violet egg medium has resulted in a gradual reversion to the R form, especially if the successive cultures have been held for some weeks. In one instance, four cultures which had been rapidly plated on gentian violet egg media for several cultural generations were transferred to tubes of the same medium. After two weeks' incubation, they were sealed and stored at room temperature for four months. On plating from the old cultures, three of the four exhibited a large proportion of R colonies, the fourth produced only R types.

The transformation from S to R may be readily followed, as has been done in other species of bacteria, through a gradual change in the form of the colony. The following series is characteristic of many similar observations. A typical S colony was isolated from a plate of a strain previously all R and which was showing mostly R types. The first subculture from this colony on Petroff's gentian violet egg medium consisted of all S types. One colony was removed and again the progeny were all S. This procedure was repeated five times with similar results. The sixth cultural generation, however, produced about an equal number of characteristic S colonies and colonies showing a raised smooth central area. Many could be only very indefinitely distinguished from the characteristic S colony, others appeared definitely as an S colony with a smaller R colony superimposed. Indeed such colonies observed from day to day at first appeared characteristically S in form and as they matured the R-like structure grew from the top. On fishing and plating the R-like tops of several such colonies various proportions of S, R and similar mixed colonies were obtained. By selecting the R colonies for several cultural generations, pure R cultures were obtained which were in every way characteristic of the original R.

Pathogenicity

Neither type of this organism has shown great pathogenicity for guinea pigs. One series of twelve guinea pigs was given subcutaneous doses of from 4 to 30 mg. of a ten-day culture of the R type grown on beef-extract glycerol broth.

Four-milligram doses produced barely palpable local nodules in seven to ten days. Seven to 30-mg. doses produced in five to seven days local nodules ranging from 5-20 mm. in diameter. The larger ones suppurated to the surface and discharged a very thick yellow exudate containing considerable numbers of acid-fast bacteria in an amorphous mass free of recognizable tissue cells. Cultures from these showed the same R types as used for inoculation. In from two to four weeks in every case the lesions were gradually resorbed; the smaller without rupturing. Several other lots of guinea pigs inoculated with other R strains produced similar results. S strains injected subcutaneously into guinea pigs in doses ranging from 5-30 mg. have produced local nodules similar to those produced by the R types. Injections made subcutaneously in the thigh have however shown a somewhat greater involvement of the lymph glands and rather more active suppuration. However, in none of the animals injected with as large doses as 30 mg. has there been any indication of generalized tuberculosis.

Similarity of R and S in Fermentation Reactions

It has generally been observed that dissociated strains within a species fail to show significant differences in fermentation reactions. Tubercle bacilli, according to Frouin and Guillaume (4) and Wherry (20) utilize glucose, maltose and galactose but not saccharose or lactose. Henley (6) on the other hand found that glucose had no marked effect upon the growth rate of tubercle bacilli.

A series of cultures of both the R and S types was made in beef-infusion broth, adjusted to pH 7.4, to which various carbohydrates were added in 1% amounts, *i.e.*, glucose, maltose, lactose, saccharose, mannite, starch and inulin. In the case of glucose-broth cultures both the R and the S cultures produced a distinctly acid reaction. With all the other carbohydrates used the cultures of both R and S remained alkaline.

Conclusions

1. It has been shown that a rapid-growing culture of presumably bovine tubercle bacilli, known to be avirulent, consists of R colony types.

2. This colony form is shown to be more or less stable on solid media and highly stable in acid-fluid media or in media low in nutrients.

3. Rapid transfers through strongly alkaline fluid media or growth in large volumes of such media result in appreciable dissociation into S types.

4. The S types were unstable and particularly on acid media reverted to the R form.

5. Neither the R nor the S types exhibited any marked degree of virulence for guinea pigs.

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THE EFFECT OF BLEACHING ON STRENGTH AND COLOR OF SASKATCHEWAN HARD RED SPRING WHEAT FLOUR[1]

By R. K. Larmour[2] and F. D. Machon[3]

Abstract

A rapid method for gas bleaching small samples has been described. A series of eight flours of varying protein content was prepared and divided into six sub-series. One of these was used as a check; three were bleached with 0.5, 1.0, and 1.5 oz. Betachlor per barrel respectively and the remaining two were treated with 0.36 and 0.72 oz. Novadel per barrel respectively. The forty-eight samples thus obtained were baked one day after treatment and again after storing for one month.

Novadel gives a good bleach but has little if any maturing effect. Betachlor is somewhat poorer than Novadel in bleaching effect but it matures the flour. The maturing effect on weak flours is slight but with high protein flours it increases with increasing dosages. This reaction seems to be dependent to some extent on the quantity of protein present.

Unbleached flour baked with addition of potassium bromate gives practically the same result as chlorine-bleached flour baked by the simple formula. Aging causes further improvement in quality of chlorine-bleached flours but not so much as in unbleached or Novadel-bleached flours. The color of the bleached samples improved with aging at almost the same rate as the unbleached samples. The blending quality of flours does not appear to improve as a result of bleaching. Both bleached and unbleached samples in this experiment when baked by the bromate formula in a blend with soft flour, gave practically the same results with respect to loaf volume, texture, and appearance, and only a slight inferiority of the unbleached in color score.

Introduction

The treatment of commercially milled flour with various bleaching reagents has become so general that it may be considered almost an integral part of the milling process at present. Its purpose is to whiten the flour by reducing the yellow pigmentation ascribed to carotin and to produce a rapid maturation simulating the effect of natural aging. It has long been known that when unbleached flour is stored, it becomes gradually whiter and at the same time undergoes a process commonly referred to as maturing or aging, whereby the baking and general handling properties of the flour improve. It is probable that in the early history of bleaching, these two processes were considered to be almost colligative, but practical experience and laboratory research have demonstrated clearly that at least in so far as artificial modification is concerned, a flour may be whitened without effecting any noticeable modification in regard to maturation. The reverse does not hold true, however, for any process that induces maturing will also effect some reduction of pigmentation.

It is not proposed to review here the extensive literature dealing with investigations of bleaching processes and their effects. Bailey (2), Ferrari and Bailey (3), Kent-Jones (6) and others have dealt very fully with this, and a

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recapitulation would serve no useful purpose. Although so much careful work has been reported, there appeared to be several phases of this problem that had been inadequately treated, and these were of immediate concern in connection with the work of this laboratory. The reasons for undertaking the investigation herein reported may be stated briefly.

Larmour and MacLeod (8), in studying various baking formulas for experimentally milled flour, observed that bleached commercial flour gave less response to $KBrO_3$ than did unbleached experimentally milled flour. It seemed probable that bromate in the baking formula plays somewhat the same role as bleaching. If this were true, the reaction to bromate would serve as an indication of the reaction to be expected from bleaching, and thereby provide a very simple and rapid means for making comparisons of wheat in respect to that particular property. The reaction to $KBrO_3$ is conditioned to a great extent by the quantity of protein present in the flour. It was considered desirable to ascertain if this is true also with regard to the action of maturing agents such as chlorine, and if the higher protein flour could be given heavier dosages without damage to strength.

Although the primary purpose of the investigation was to study the effect of bleaching agents on the strength of the flour, it was also realized that reaction to bleaching is very important. An extensive program of plant breeding for rust and disease resistance is in progress, and in many instances one of the progenitors of a cross is highly pigmented. In many cases the principal determining factor in making final selections is color of flour produced. There arise the questions; can the pigmentation of the flour be reduced to an acceptable level without damaging the bread-making quality of the flour; can certain flours be more easily bleached than others? Adequate means for making thorough tests of reaction to bleaching and the effect of bleaching on strength of flour must be employed if the cereal chemist is to be in a position to give sound judgments on the materials submitted by the plant breeder.

Small scale bleaching methods described in the literature are very time-consuming and for that reason seemed to be inapplicable to the routine of the wheat-testing laboratory. Accordingly, the development of a bleaching method that would combine a fair degree of accuracy with considerable rapidity of application was undertaken.

Method of Bleaching

Chlorine and Novadel were selected as bleaching agents for this study, because they are said to be fairly typical of the two types in common use. The work of James and Huber (5), Ferrari and Bailey (3) and others has shown that treatment with chlorine gives improvement of loaf volume as well as bleaching. Novadel, the active principal of which is benzoyl peroxide, produces an excellent bleaching effect without any pronounced change in loaf volume. The former may be considered typical of the class that matures and bleaches, while the latter is typical of the class that bleaches only.

Two principal difficulties are encountered in attempting to carry out routine bleaching in the laboratory, namely, the standardization of the dosage and the time and attention that must be given to the procedure, the latter being probably the more important. The method should be rapid, or if this is not possible, should be nearly automatic.

In the application of Novadel, the dosage can be very accurately standardized by careful weighing. In this work 77 mg. per 600 gm. of flour was used as normal. This corresponds to 1 lb. per 45 barrels. The problem with Novadel is to get the powder uniformly mixed with the flour. To accomplish this, an earthenware jar made by the Ceramics Department of this university was used. This jar was of one gallon capacity, 9½ in. in diameter and 10 in. high, made to fit the standard ball-mill equipment. The top was of the pickle-jar type with a spring clamp fastener. The shoulders were smoothly curved up to the opening, to permit easy and rapid cleaning. Inside, two 1½-inch baffle plates extending the length of the jar were built in at an angle of about 20° to the horizontal axis. The inside was highly glazed. When this jar is slowly rotated in the ball-mill frame, the flour spills over these baffle plates and also shifts slowly back and forth. Very thorough mixing of the flour is thus ensured. For incorporating Novadel or other powders with flour, the apparatus is rotated for 20-25 min. As it is motor driven, the only attention necessary is that involved in loading and unloading. By weighing out all the required portions of Novadel before starting, a man could operate the experimental mill and do the necessary Novadel bleaching as the flours are obtained.

For chlorine bleaching, the methods used by James and Huber (5) or by Ferrari and Bailey (3) are too slow and require too much attention for use in routine testing. It would require one man's full time to handle a daily output of the experimental mill. The amazing rapidity with which flour absorbs chlorine led to the hope that a very much faster process could be developed and accordingly an attempt was made to imitate as far as feasible the commercial procedure of treating a continuous flow of flour with a continuous flow of chlorine mixed with air. After some experimenting, the apparatus shown in Fig. 1 was found to work quite satisfactorily.

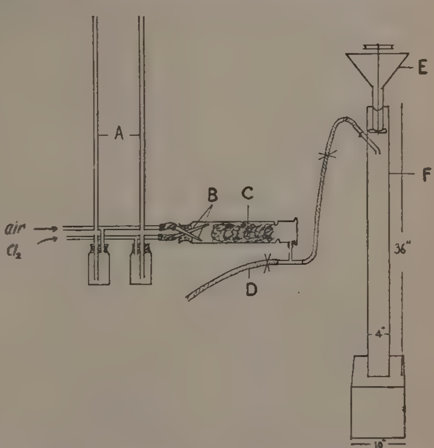

FIG. 1. *Diagram of apparatus for chlorine bleaching. A, oil manometers; B, fine glass jets; C, loosely packed glass wool; D, by pass to hood; E, flour distributor; F, wooden pipe 2½" inside cross section.*

In this procedure it was necessary to synchronize the rate of flow of gas with the rate of flow of flour. The rate of delivery of chlorine and air was quite

simply controlled by use of two oil manometers and a pair of small glass jets. Three volumes of air to one of chlorine were used. For mixing, the gases were passed into a soda-lime tower partly filled with loosely packed glass wool. The chlorine jet was directed toward the top of the vessel, and the air jet, terminating a little behind the other, was directed downward. It was assumed that the gases were quite well mixed by the time they had passed through the glass wool. The mixed gases were led off from the lower side of the vessel as shown in the figure, into a T-tube that provided a by-pass to the hood when bleaching was not in progress. The manometers were standardized by displacement of saturated sodium chloride solution. Air was obtained directly from the compressed air line and chlorine from a cylinder. When routine bleaching was in progress, the respective gas pressures were adjusted and thereafter until the day's run was finished, they were left, the gases being diverted to the hood when not required.

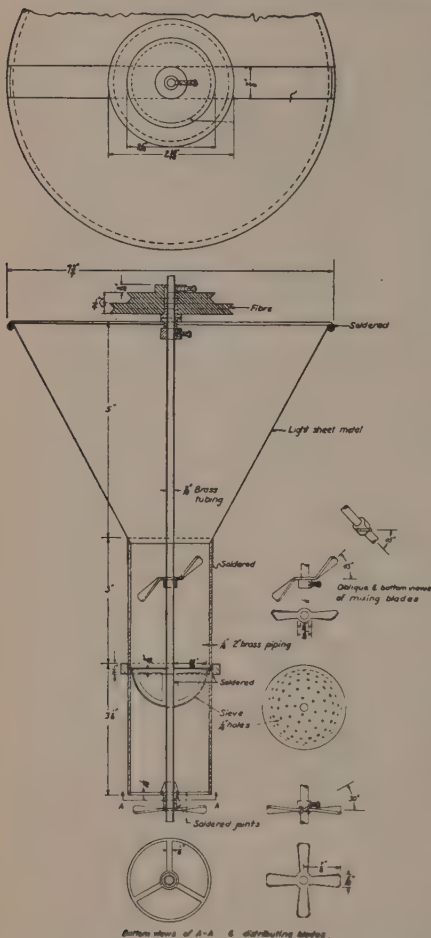

FIG. 2. *Details of construction of the flour distributor.*

The flour distributor, constructed in the university instrument shop, consisted essentially of a spun brass bowl with perforations and a horizontally rotated stirring arm. A working diagram of this device is shown in Fig. 2. The four-bladed fan at the lower end of the vertical drive shaft was designed to spread the flour stream so that it might occupy most of the cross-section of the bleaching chamber—Fig. 1. The two-bladed fan on the shaft above the bowl was found necessary to prevent the flour clogging in the throat of the tube. The apparatus was designed to handle 600 gm. of flour at one time.

In operation, the motor speed was adjusted by means of a rheostat to deliver 300 gm. of flour per minute. The gas pressures were set to deliver 30 cc. of chlorine and 90 cc. of air per minute. This is equivalent to a chlorine dosage of approximately 0.5 oz. per barrel. In routine work, standard batches of 600 gm. of flour were used. A sample was first put through the distributor

several times to check the rate of flow, and any adjustments of motor speed necessary were made. The batch was then placed in the hopper, the gas flow diverted to the bleaching tube and the motor switched on. The gas flow was checked closely against the watch and shut off at the end of two minutes. It is advisable to regulate the flour flow to a few seconds past the two minutes in order that the last traces of chlorine may be swept out of the bleaching tube. This requires close and careful attention but as the whole procedure, including weighing of the flour, takes only about five minutes, one can afford to watch it.

Materials and Methods

The material studied consisted of eight samples of pure strain Marquis grown in different parts of Saskatchewan from first generation seed sent out by this department. These were selected on the basis of protein content from fifty such samples, to give a range representative of the 1929 crop. A description of the samples is given in Table I.

TABLE I

ANALYTICAL AND MILLING DATA

Flour No.	Grade	Wt. per bu., as received	Protein % (13.5% moisture basis)		Milling data		
			Wheat	Flour	Flour %	Total flour %	Straight as % of total
1	3 Nor. starchy	63.5	8.8	8.0	68.0	73.1	93.0
2	2 Nor. starchy immature	63	10.7	10.0	71.4	75.9	94.1
3	3 Nor. pink green	61	11.7	11.0	68.3	73.1	93.4
4	2 Nor. immature	60	12.7	12.3	69.5	73.6	94.4
5	1 hard	64.5	13.6	12.9	72.0	76.1	94.6
6	1 hard	65	14.6	13.6	72.8	76.7	94.9
7	3 Nor. shrunken	56	16.1	16.0	67.2	70.4	95.4
8	2 Nor. shrunken	58	18.2	17.6	69.0	72.9	94.6

Three millings of straight flour were made on each, the flour was thoroughly mixed and 600-gm. portions were weighed out and treated immediately with the following dosages of bleaching agent. A—unbleached check sample; B—0.5 oz. Betachlor per barrel; C—1.0 oz. Betachlor per barrel; D—1.5 oz. Betachlor per barrel; E—0.36 oz. Novadel per barrel; F—0.72 oz. Novadel per barrel.

Bakings were made the day following the bleaching and after storing one month in cotton sacks. The time and temperature of fermentation and baking used were the same as recommended by the Baking Committee of the A.A.C.C., but the rest of the procedure differed somewhat. Variable absorption to suit the flour, machine mixing, low-sided baking tins, and formulas involving 50 gm. of flour only, were the departures from the standard method. Three formulas have been used. Formula I referred to as the "basic" formula was flour 50 gm. (13.5% moisture basis), yeast 3%, salt 1%, sugar 2½%, water as required. In formula II, referred to as the "bromate" formula, 0.001% potassium bromate was added to the above mentioned ingredients. In formula III the ingredients were as in formula II except that in place of 50 gm. of straight flour, 25 gm. was mixed with an equal amount of pastry flour milled from Pacific

TABLE II

GASOLINE COLOR VALUES OF THE VARIOUS SAMPLES TREATED

Treatment	Samples								
	1	2	3	4	5	6	7	8	Average
Gasoline color values									
A. Unbleached	1.59	1.35	1.40	1.25	1.25	1.34	1.27	1.30	1.34
B. 0.5 oz. Betachlor per bbl.	0.98	1.00	0.91	0.89	0.81	0.83	1.07	0.97	0.93
C. 1.0 oz. Betachlor per bbl.	0.91	0.72	0.83	0.68	0.67	0.64	0.86	0.74	0.75
D. 1.5 oz. Betachlor per bbl.	0.79	0.63	0.67	0.61	0.40	0.56	0.65	0.61	0.62
E. 0.36 oz. Novadel per bbl.	1.03	0.88	0.91	0.81	0.67	0.67	0.94	0.83	0.84
F. 0.72 oz. Novadel per bbl.	0.65	0.50	0.53	0.43	0.37	0.39	0.52	0.46	0.48
Reduction in gasoline color value, %									
A. Unbleached									
B. 0.5 oz. Betachlor per bbl.	38	26	35	29	35	38	16	25	30
C. 1.0 oz. Betachlor per bbl.	43	47	41	46	46	52	32	43	44
D. 1.5 oz. Betachlor per bbl.	50	53	52	51	68	58	49	53	54
E. 0.36 oz. Novadel per bbl.	35	35	35	35	46	50	26	36	37
F. 0.72 oz. Novadel per bbl.	59	63	62	66	70	71	59	65	64

soft wheats. This will be referred to as the "blend" formula. The 50-gm. formula was used because the amount of flour available was small and at least five bakings in duplicate were desired. Larmour and MacLeod (8) found that the 50-gm. loaves give relative results similar to the 100-gm. loaves.

To ascertain the effectiveness of the bleaching, the gasoline color value was determined by the method outlined by the Association of Official Agricultural Chemists (1). The results are shown in Table II. For economy of space, only the gasoline numbers of the unbleached check samples have been recorded, the rest being shown as percentages of the corresponding check sample.

These values show considerable variability, part of which is doubtlessly attributable to manipulative errors. There is however evidence of distinct specificity in reaction to the bleaching agents. Samples 4, 5 and 7, for instance, show practically the same color for the unbleached flour, but each reacts differently to both the Betachlor and the Novadel. Sample 7 responds least and sample 5 responds most to bleaching. These differences can be seen in all the treatments and therefore can scarcely be attributed to errors in the dosage.

Comparing the Novadel bleach with the Betachlor, the former gives the greatest reduction of color for the dosages used. Application of Novadel at the rate of 0.36 oz. per barrel appears to give about the same results as would be estimated for a dosage of $\frac{3}{4}$ oz. Betachlor.

The various samples prepared as described were baked by the basic and bromate formulas on the day after they were bleached, and again after they had aged in cotton sacks for one month. In this second baking the blend formula was also used. For the purpose of the discussion, the various bakings will be considered separately.

Immediate Effect of Bleaching on Baking Quality

The immediate effect of various dosages of bleaching agents may be estimated best from the baking done 24 hr. after treatment, as the modifications due to natural aging and bleaching would introduce the least error at that time. The baking data obtained by the basic method on the day after the bleaching was done, are shown in Table III.

Considering first the loaf volume, which may be taken as a measure of the apparent strength of the flour, it is seen that the two lowest protein flours, No. 1 and 2, show little evidence of a maturing effect with any of the dosages. No. 1B shows a substantial increase over the check sample but as the 1D is of practically the same value as the check, it might be assumed that the value 261 is the result of manipulative errors. All of the other flours show evidence of aging as a result of the Betachlor treatment. No. 3 and 4 appear to be as much matured with the $\frac{1}{2}$-oz. dosage as with the $1\frac{1}{2}$-oz. dosage. Increasing the bleach with these produces practically no increase of volume. With the remaining four flours, there is an increase of volume with increasing dosage of chlorine, with the possible exception of 6C and 6D, where the difference 6 cc. is probably not significant.

TABLE III

THE EFFECT OF BLEACHING ON BAKING QUALITY AS SHOWN BY THE BASIC METHOD; BAKED 24 HR. AFTER BLEACHING

Flour No.	Sample No.	Absorption %	Appearance of loaf	Color of crumb	Texture of crumb	Loaf volume cc.	Baking score	Loaf volume as % of A	Baking score as % of A
1	A	66	6.5	4.5	5.5	246	56	100	100
	B	66	6.5	5.5	6	261	66	106	118
	C	66	6.5	5.5	6	238	57	97	102
	D	66	6.5	7	7.5	241	65	98	116
	E	66	7	5	6.5	242	59	98	105
	F	65	7	5.5	6	242	57	98	102
2	A	68	9	5	5	260	66	100	100
	B	68	9	6	6.5	257	71	99	108
	C	68	9	6.5	7	263	76	101	115
	D	68	9	7.5	7.5	265	78	102	118
	E	68	9	6	5	245	62	94	94
	F	68	9	6.5	6	245	66	94	100
3	A	69	9	5	4.5	263	67	100	100
	B	69	9	6	5.5	277	77	105	115
	C	69	9	6.5	6	278	80	106	119
	D	69	9	7.5	6.5	283	86	108	128
	E	69	9	5.5	4	264	67	100	100
	F	69	9	6	4.5	272	72	103	107
4	A	65	9.5	6	7.5	250	69	100	100
	B	65	9.5	7	8.5	285	88	114	127
	C	65	9.5	8	8.5	289	92	116	133
	D	65	9.5	9	9	284	93	114	135
	E	65	9	6.5	8	250	71	100	103
	F	65	9	7	7.5	248	70	99	101
5	A	68	10	6.5	5	285	80	100	100
	B	68	10	7.5	6	320	99	112	124
	C	68	10	8.5	7	348	115	122	144
	D	68	10	9.5	8	357	124	125	155
	E	68	10	6	6	283	81	99	101
	F	68	10	7	7	288	88	101	110
6	A	69	8	6	5.5	283	79	100	100
	B	69	8	7	6.5	293	88	103	111
	C	69	8	8	7	308	97	109	123
	D	69	8	9	8	314	105	111	133
	E	69	8	7	6	283	82	100	104
	F	69	8	8.5	6.5	289	89	102	113
7	A	68	8.5	5	6	278	76	100	100
	B	68	9	6	6	288	82	104	108
	C	68	9	7	7	300	92	108	121
	D	68	9.5	8	7	310	98	111	129
	E	68	8.5	5.5	6	277	76	100	100
	F	68	9	7	7.5	282	86	101	113
8	A	68	7	5	7.5	271	76	100	100
	B	68	8	6	7.5	282	83	104	109
	C	68	9	6.5	7.5	293	90	108	118
	D	68	9	7	7.5	313	99	115	130
	E	68	7.5	6	7	275	78	101	103
	F	68	8.5	6.5	8	279	85	103	112

These data lead to the conclusion that with respect to apparent strength, the reaction to chlorine is directly related to the protein of the flour. Very low protein flours show no beneficial result; the medium protein flours probably give maximum results with small dosages, while the higher protein flours exhibit responses to progressively greater dosages of chlorine.

The samples of the E and F subgroups, bleached with Novadel, show only slight deviations from the values of the checks, and the differences scarcely can be considered significant. It may be concluded therefore that Novadel produces no immediate maturing effect that can be detected by the basic method.

Examining the other loaf characteristics, the outside appearance shows little effect of the bleaching, except in the case of flour No. 8, in which there is quite a distinct improvement with increasing dosage of chlorine. Crumb color, as would be expected, improves with bleaching. The texture scores indicate improvement with increasing chlorine treatment, except in the case of flour No. 8, which shows no change in this respect. For the greater part, the improvement of texture as a result of Novadel is of doubtful significance in this baking.

As the baking score* is calculated from all of these characteristics, it may be considered a measure of general bread-making quality. With respect to this value, flour No. 1 shows the least change and No. 5 the greatest, as a result of bleaching. With the exception of No. 1 which gives irregular results, all exhibit an increase with increasing dosages of chlorine. With the Novadel bleach, all show no significant difference for the E dosage. The first four exhibit little difference, but the last four all give higher results with the F dosage.

From this baking the following conclusions may be drawn regarding the effect of the various dosages of bleaching agents used:

Novadel produces no significant changes in loaf volume in the series and none in the baking score except in the four highest protein flours, in which cases there seems to be distinct evidence of improvement of baking quality with the heavier dosage, namely, 0.72 oz. per barrel.

Betachlor affects the loaf volume of the lowest protein flours least and the higher protein flours most. As far as these baking data go it seems as though the reaction to bleaching is conditioned by the amount of protein.

Bromate Baking Twenty-four Hours After Bleaching

The bromate data given in Table IV reveal some characteristics of the flour not apparent in the basic data. For instance, the loaf volume of the two weak flours, 1 and 2, shows a distinct decline for the C and D dosages of chlorine, indicating probably that after one day's aging, the 0.5 oz. per barrel dosage

* *Calculation of baking score:—*
(Loaf volume minus 200) $\times$*0.4*
Texture $\times$*3*
Crumb color $\times$*2*
Appearance $\times$*1*
(Absorption minus 60) $\times$*1*
Baking score = sum of these.

TABLE IV

THE EFFECT OF BLEACHING ON BAKING QUALITY AS SHOWN BY THE BROMATE METHOD; BAKED 24 HR. AFTER BLEACHING

Flour No.	Sample No.	Absorption %	Appearance of loaf	Color of crumb	Texture of crumb	Loaf volume cc.	Baking score	Loaf volume as % of A	Baking score as % of A
1	A	68	6	5.5	6.5	258	67	100	100
	B	68	6	6.5	7.5	256	71	99	106
	C	68	6	6	7	234	60	91	89
	D	68	6	6.5	6.5	232	58	90	87
	E	68	6	6	7.5	249	67	96	100
	F	68	6	6.5	7	243	64	94	95
2	A	69	8	5.5	7	279	79	100	100
	B	69	8	7	8	286	89	102	113
	C	69	8	8	8	264	80	95	101
	D	69	8	7	7.5	259	76	93	96
	E	69	8	6.5	7.5	275	81	99	102
	F	69	8	8	8	275	86	99	109
3	A	69	8	6.5	9	324	109	100	100
	B	69	9	7.5	9	333	114	103	105
	C	69	9	8.5	9	338	118	104	108
	D	69	9	9	9	335	118	103	108
	E	69	9	6.5	8.5	315	104	97	95
	F	69	9	7.5	9	310	105	96	97
4	A	69	8	7	9	296	94	100	100
	B	69	8	8	8.5	310	100	105	106
	C	69	8	9	9	315	106	106	113
	D	69	8	10.5	8.5	312	110	105	117
	E	69	8	7.5	8.5	301	95	102	101
	F	69	8	8.5	8.5	305	99	103	105
5	A	69	10	7	7.5	388	129	100	100
	B	69	10	8	7.5	377	126	97	98
	C	69	10	9	8	358	122	92	95
	D	79	10	9	7	379	128	98	99
	E	69	9	7	8	347	114	89	88
	F	69	8	8	8	360	121	93	94
6	A	71	8	6.5	8.5	360	122	100	100
	B	71	8	7.5	8	379	130	105	106
	C	71	9	9	8	388	136	108	111
	D	71	10	9.5	8.5	394	141	109	116
	E	71	9	7.5	9.5	353	124	98	102
	F	71	9	9	9.5	372	134	103	110
7	A	70	8	7	7.5	360	118	100	100
	B	70	8	8	8	375	128	104	108
	C	70	8	9	7.5	390	134	108	113
	D	70	8	10	8	391	138	109	117
	E	70	8	7.5	8	359	121	100	102
	F	70	8	8.5	8	345	117	96	99
8	A	69	8	6	8	338	108	100	100
	A	69	8	7	7	367	119	109	110
	C	69	8	8	6.5	372	121	110	112
	D	69	10	9	6	389	128	115	118
	E	69	8	6.5	8.5	348	114	103	105
	F	69	7	7.5	8.5	340	113	101	105

is not injurious to strength, and that the 1-oz. and 1.5-oz. dosages effect a diminution of strength. Flours 3, 4 and 5 show practically no differences of loaf volume with the three dosages of chlorine, and flours 6, 7 and 8 all show increased loaf volume with increasing amounts of chlorine.

With the Novadel, three flours show differences with the two dosages. No. 5 and 6 gave increased loaf volume with increase of the bleaching agent, while No. 7 gave a slight decrease. All the others exhibit no differences between the two bleaches and in fact no difference from the loaf volume of the check samples.

The crumb color in general improves with increasing dosage of the bleaching agents. The texture however is only slightly, and probably not significantly higher than that of the check samples. It will be recalled that with the basic baking, a decided improvement of texture resulted from the treatment with chlorine, and in some cases also from the treatment with Novadel, except in the case of the highest protein flour. This differentiation of texture disappears in the bromate data, indicating that to some extent the effect of the potassium bromate on the untreated flour is similar to the effect of the chlorine.

With the baking scores, the differentiation is much less marked than in the basic data. For flours 1, 2, 3 and 5 the differences between the various treatments and the respective check samples are small, irregular and probably not significant. With the other four flours there does appear to be a progressive increase of baking score from A to D. With the exception of 6F, there is no significant difference from the check sample attributable to the Novadel bleaches.

One of the purposes of the experiment was to ascertain if the action of bromate is similar to the action of maturing agents. A comparison of the results obtained with the check sample by the bromate formula, with the best corresponding bleached sample by the basic formula, should throw some light on this question. For convenience these data have been collected in Table V.

For flour No. 1 the results are practically identical. This is to be expected, as it is so weak that neither bromate nor bleaching nor, as will be seen later, natural aging produce any improvement. Flours 2, 4 and 5 give results that may be considered not significantly different, although, in each case, the check bromate results are slightly higher. In all the other samples the bromate gives higher values on the unbleached sample than does the basic method on the bleached sample. With the exception of flour 3 (from 11.7% protein wheat) flours of medium and low protein content react to bromate to give baking results similar to those obtained with artificially matured flours. The higher protein flours however appear to react better to the influence of bromate than to the chlorine. This might indicate that the higher protein flours could be improved further by heavier dosages of the bleaching agent.

To test this postulate, the residue of the check samples was divided into two portions and these were bleached with dosages of 2.0 oz. and 2.5 oz. Betachlor per barrel respectively. These subsamples will be called G and H. They cannot be considered strictly comparable to the other subsamples as the flour had aged for nearly eight weeks before these treatments were made. The writers were particularly interested however in finding how the higher protein

flours would react, in order to make an approximate test of the conclusion indicated by the data at hand. The results with the subsamples treated with 2.5 oz. Betachlor per barrel are shown in parentheses in Table V.

TABLE V

COMPARISON OF UNBLEACHED SAMPLES BAKED BY THE BROMATE FORMULA AND BLEACHED SAMPLES BAKED BY THE BASIC FORMULA

Flour No.	L.V. bromate check samples	L.V. basic bleached samples	C.B.S. bromate check samples	C.B.S. basic bleached samples	Treatment of bleached samples
1	258	261 (225)	67	66 (36)	B. 0.5 oz. chlorine
2	279	257 (240)	79	71 (61)	B. 0.5 oz. chlorine
3	324	283 (290)	109	86 (94)	D. 1.5 oz. chlorine
4	296	289 (290)	94	92 (92)	C. 1.0 oz. chlorine
5	388	357 (365)	129	124 (129)	D. 1.5 oz. chlorine
6	360	314 (350)	122	105 (125)	D. 1.5 oz. chlorine
7	360	310 (335)	118	98 (119)	D. 1.5 oz. chlorine
8	338	313 (360)	108	99 (121)	D. 1.5 oz. chlorine

NOTE:—*Values in parentheses were obtained on subsamples H treated with 2.5 oz. Cl_2 per bbl.*

While certain reservations on account of differences in age at time of bleaching must be made when comparing these values with those of the main series, one can quite legitimately compare the H subsamples by themselves. They show that flours 1 and 2 were seriously over-bleached by this treatment; flours 3, 4, 5 and 6 underwent very little change; flours 6, 7 and 8 reacted favorably, giving marked improvement. These last three improved to the extent that the values by the basic method were virtually equal to or better than the values obtained by the bromate method on the unaged unbleached samples. If any weight can be attached to this H group of samples, the conclusion might be reached that the data obtained by baking the unbleached, freshly milled flour by the bromate formula would in general be indicative of the results to be expected from flours fully matured by artificial means baked without addition of bromate.

Summary of Conclusions from the First Bakings

The information derived from these two initial bakings may be summarized as follows.

No evidence that Novadel treatment results in increase of loaf volume was obtained from either the basic or bromate bakings. This bleaching agent produced a marked reduction of color but no maturing effect.

The effect of the Betachlor on the two lowest protein flours was negligible. The flours of medium protein content showed slight increase of loaf volume by the basic formula, with increasing dosage, but this differentiation for the most part disappeared in the bromate data. The three flours 6, 7 and 8 of highest protein content, by both methods showed distinct increases of loaf volume with increasing dosages of the bleaching agent. This indicates that the reaction to Betachlor is conditioned by the amount of protein present.

TABLE VI

THE EFFECT OF BLEACHING ON BAKING QUALITY AS SHOWN BY THE BASIC METHOD ONE MONTH AFTER BLEACHING

Flour No.	Sample No.	Basic baking data							
		Absorption %	Appearance of loaf	Color of crumb	Texture of crumb	Loaf volume cc.	Baking score	Loaf volume as % of A	Baking score as % of A
1	A	68	6	4	3.5	235	46	100	100
	B	68	6	5	4	247	55	105	120
	C	68	6	6	4	232	51	99	111
	D	68	6	5	4	235	50	100	109
	E	68	6	5	3.5	240	50	102	109
	F	68	6	5	4	240	52	102	113
2	A	69	8	8	7	257	77	100	100
	B	69	8	7	7.5	272	82	106	106
	C	69	8	7.5	8	270	84	105	109
	D	69	8	8.5	8	266	84	104	109
	E	69	8	7	7	257	75	100	97
	F	69	8	7.5	7.5	257	77	100	100
3	A	69	8	5	5	267	69	100	100
	B	69	9	7	6	282	83	106	120
	C	69	9	6.5	6	275	79	103	114
	D	69	9	8	6.5	282	86	106	125
	E	69	9	6	5.5	267	73	100	106
	F	69	9	6.5	6	272	78	102	113
4	A	69	8	7.5	8.5	280	90	100	100
	B	69	8	8.5	8.5	315	106	113	118
	C	69	8	9	8.5	305	102	109	113
	D	69	8	10	9.5	312	110	111	122
	E	69	8	8.5	8	285	92	102	102
	F	69	8	9	8	280	91	100	101
5	A	69	10	8	8	308	102	100	100
	B	69	10	9	8	342	118	111	116
	C	69	10	10	9	362	131	118	128
	D	69	10	11	9	367	135	119	132
	E	69	9	9	8.5	307	104	100	102
	F	69	8	9.5	9	322	112	105	110
6	A	71	8	6.5	7	290	89	100	100
	B	71	8	7.5	8	305	100	105	112
	C	71	9	8.5	8.5	310	106	107	119
	D	71	10	9.5	9.5	332	121	114	136
	E	71	9	8	7	282	90	97	101
	F	71	9	9.5	8	287	98	99	110
7	A	70	8	6	6	292	85	100	100
	B	70	8	7	7.5	317	101	109	119
	C	70	8	8	7.5	322	105	110	124
	D	70	8	9.5	8.5	340	118	116	139
	E	70	8	8	7.5	302	97	103	114
	F	70	8	7.5	6.5	282	85	97	100
8	A	69	8	6.5	8	295	92	100	100
	B	69	8	7.5	8.5	312	102	106	111
	C	69	8	8	8	317	104	107	113
	D	69	10	9	8	335	115	114	125
	E	69	8	8	8.5	290	94	98	102
	F	69	7	9	9	290	97	98	105

There is evidence that the effect of bromate on a flour is similar to the effect of the Betachlor. The unbleached, unaged check samples baked with the addition of 0.001% $KBrO_3$ gave results comparable to those obtained with the artificially matured samples baked by the basic method.

Baking Results Obtained After Aging

After the initial bakings, the samples were placed in cotton sacks and stored in the mill room for one month. The temperature of this room averages about 18° C. and it is kept at approximately 75% relative humidity during the day, by means of a Bahnson humidifier. Thereafter they were baked in duplicate by the basic, bromate and blend formulas using the procedure described above.

Tables VI and VII give the complete data obtained with the second baking by the basic and bromate formulas. With regard to the relative effect of the different dosages of bleaching agents used, these data confirm the conclusions drawn from the initial bakings, and in this respect furnish little additional information. The two sets of baking data do however provide means for making a comparison of the effect of bleaching on the rate of improvement due to natural aging.

On account of the difficulty of referring to such large tables it has seemed advisable to retabulate, using loaf volumes and baking score as the chief characteristics on which to base the comparisons. In Table VIII the percentage increase of loaf volume due to aging is shown.

Comparing first the various subsamples by means of the averages, there seems to be little significant difference. This indicates on the average the same increase in loaf volume, or in other words, that the bleached samples aged relatively to the same extent as the unbleached samples.

It is interesting to note that flour No. 1, the lowest in protein, showed unmistakable evidence of a decrease in strength on aging. Flours 4, 6, 7 and 8 gave the largest increase.

Turning next to the data obtained with the bromate formula, it is seen that here again flour No. 1 shows evidence of deterioration. No. 3 shows decrease in loaf volume of the check, and of the C and D subsamples. As the B subsample showed an increase, it seems probable that the differences may be due to experimental error. On the average, the samples bleached by the Betachlor show slightly less, and those bleached by Novadel slightly more improvement in the loaf volume by the bromate method, than the unbleached check samples.

Table IX gives the percentage increase of baking score due to aging. As all of the characteristics of the bread are considered in this score, it serves as a measure of general bread-making quality of the flour. In general the percentage increases are much larger than in the case of the loaf volumes. This is due partly to the influence of crumb color which improves very considerably with age. This applies to the bleached as well as the unbleached samples.

On account of the great variability of these values, it is difficult to draw conclusions regarding the specific subsamples. It seems evident however that in general the C and D samples, treated with 1.0 oz. and 1.5 oz. of Betachlor

TABLE VII

THE EFFECT OF BLEACHING ON BAKING QUALITY AS SHOWN BY THE BROMATE METHOD BAKED ONE MONTH AFTER BLEACHING

Flour No.	Sample No.	Absorption %	Appearance of loaf	Color of crumb	Texture of crumb	Loaf volume cc.	Baking score	Loaf volume as % of A	Baking score as % of A
1	A	68	8.5	5.5	6.5	232	60	100	100
	B	68	8.5	6.5	6.5	235	63	101	105
	C	68	8.5	7	6.5	230	62	99	103
	D	68	8.5	7.5	6.5	230	63	99	105
	E	68	8.5	7.5	6.5	242	68	104	113
	F	68	8.5	8	6.5	240	68	103	113
2	A	69	10	7.5	8	302	99	100	100
	B	69	10	8.5	8	297	99	98	100
	C	69	10	9	8	275	91	91	92
	D	69	10	9	7.5	275	90	91	91
	E	69	10	8	8	290	95	96	96
	F	69	10	9.5	9	305	107	101	108
3	A	71	10	8	8.5	317	109	100	100
	B	71	10	9	8.5	342	121	108	111
	C	71	10	9.2	9	332	119	105	109
	D	71	10	9.7	9	325	117	102	107
	E	71	10	8	9	330	116	104	106
	F	71	10	9	8	310	100	98	92
4	A	69	10	8.5	8.5	322	110	100	100
	B	69	10	9.5	8.5	332	116	103	105
	C	69	10	9.7	9	317	112	98	102
	D	69	10	10.5	8.5	312	110	97	100
	E	69	10	9	8	317	108	98	98
	F	69	10	10.5	8	317	111	98	101
5	A	69	10	8.5	6.5	405	138	100	100
	B	69	10	9.2	7.5	390	136	96	98
	C	69	10	10	7.5	387	136	95	98
	D	69	10	11	7.5	395	142	97	103
	E	69	10	9	7.5	392	136	97	98
	F	69	10	10	8	395	141	97	103
6	A	71	10	9.7	7.5	390	139	100	100
	B	71	10	10.5	8	387	141	99	101
	C	71	10	10	8	395	143	101	103
	D	71	10	11	8	382	140	98	101
	E	71	10	8.5	8	365	128	94	92
	F	71	10	9.2	9	377	137	97	98
7	A	70	10	8	8	365	126	100	100
	B	70	10	8.7	7.5	380	132	104	105
	C	70	10	9	8	395	140	108	111
	D	70	10	10	7	405	143	111	113
	E	70	10	9	8	387	137	106	109
	F	70	10	9.5	7.5	380	134	104	106
8	A	69	10	7	5.5	377	120	100	100
	B	69	10	8	5.5	382	124	101	103
	C	69	10	8.7	5	390	127	103	106
	D	69	10	9.5	5	405	135	107	112
	E	69	10	8	6	385	127	102	106
	F	69	10	9	6.5	395	132	105	110

respectively, show less improvement due to aging than any of the others. This holds for both baking methods. From the last column of Table VII it can be seen that the baking scores of subsamples C and D are in general equal to or greater than the baking scores of the other subsamples. This taken in conjunction with the observation that the percentage increase of baking scores for the subsamples C and D is less than for the others, leads to the conclusion that the heavier treatment received by these samples produced an initial maturity closer to the maximum than in the other cases.

TABLE VIII

Increases in loaf volume after aging for one month

Flour No.	L. V. by basic formula percentage increase						Mean	L. V. by bromate formula percentage increase						Mean
	A	B	C	D	E	F		A	B	C	D	E	F	
1	−5	−5	−2	−3	−1	−1	−3	−10	−8	−2	−1	−3	−1	−4
2	−1	6	3	0	5	5	3	8	4	4	6	5	11	6
3	1	2	−1	0	1	0	1	−2	3	−2	−3	5	0	0
4	12	10	5	10	14	13	11	9	7	1	0	5	4	4
5	8	7	4	3	8	12	7	4	3	8	4	13	10	7
6	2	4	1	6	0	−1	2	8	2	2	−3	3	1	2
7	5	10	7	9	9	0	7	1	1	1	4	8	10	4
8	9	11	8	7	5	4	7	11	4	5	4	11	16	8
Mean	4	6	3	4	5	4	4.4	4	2	2	1	6	6	3.5

TABLE IX

Increases in baking score after aging for one month

Flour No.	Basic percentage increases						Mean	Bromate percentage increases						Mean
	A	B	C	D	E	F		A	B	C	D	E	F	
1	−8	−17	−11	−23	−15	−9	−14	−10	−11	3	9	1	6	0
2	17	15	10	8	21	17	15	25	11	14	18	17	24	16
3	3	8	−1	0	9	8	4	0	6	1	−1	12	−5	2
4	30	20	11	18	30	30	23	17	16	6	0	14	12	11
5	28	19	14	9	28	27	21	7	8	11	11	19	16	12
6	13	14	9	15	10	10	12	14	8	5	−1	3	2	5
7	12	23	14	20	28	−1	16	7	3	4	4	13	14	8
8	21	23	16	16	20	14	18	11	4	5	5	11	17	9
Mean	14.5	13	8	8	13	12	11.5	9	8	6	6	11	11	8.6

Effect on Crumb Color of Natural and Artificial Bleaching

The relative effect of artificial and natural bleaching on crumb color may be estimated by comparing the crumb colors obtained with the unbleached aged flours, and those obtained with the bleached unaged flours. These data have been collected in Table X. There are also shown the color scores of the bleached

samples after aging. The scores obtained from the bromate baking have been used because the finer texture of the bread baked by this formula enables one to judge the crumb color more accurately than when the basic formula is used.

These data show that the 1.5-oz. dosage of Betachlor gives in general an immediate improvement in color greater than that resulting from one month's aging of the unbleached sample. The 0.72-oz. dosage of Novadel produces an immediate effect practically equal to that resulting from one month's aging of the unbleached samples. Perhaps a more interesting observation is that even with this marked initial improvement of color, the process of aging effects a further improvement of the treated samples, in most cases as great as that which occurs in the unbleached flours.

TABLE X
Comparison of artificial and natural bleaching
Crumb color scores by bromate formula

Flour No.	A. Unbleached		D. Bleached with 1.5 oz. Betachlor		F. Bleached with 0.72 oz. Novadel per barrel	
	Aged 1 day	1 month	Aged 1 day	1 month	Aged 1 day	1 month
1	5.5	5.5	6.5	7.5	6.5	8
2	5.5	7.5	7	9	8	9.5
3	6.5	8	9	9.7	7.5	9
4	7	8.5	10.5	10.5	8.5	10.5
5	7	8.5	9	11	8	10
6	6.5	9.7	9.5	11	9	9.2
7	7	8	10	10	8.5	9.5
8	6	7	9	9.5	7.5	9

Summary of Observations on Aging

With all the subsamples used, it was found that an improvement in loaf volume and color occurred as a result of aging. The improvement of loaf volume was least with those samples that showed the greatest evidence of maturing immediately after bleaching, namely the C and D subsamples. With the exception of the weakest flour, No. 1, the samples in general, irrespective of the color immediately after bleaching, showed a marked improvement of color as a result of aging one month.

The Effect of Bleaching on the Blending Value of Flour

As a large proportion of the wheat grown in Western Canada is exported to Europe, and there mixed with softer wheats, the reaction to blending is of very great importance. The behavior of a sample of flour when mixed with very weak flour should provide a fairly good estimate of its value to the European buyer. For this reason, a blend baking formula has been included as part of our testing routine.

In the blend formula there is used 40% of Pacific Club wheat flour and 60% of the flour under test and the mixture is baked by the bromate formula.

TABLE XI

THE EFFECT OF BLEACHING ON BAKING QUALITY AS SHOWN BY THE BLEND METHOD BAKED ONE MONTH AFTER BLEACHING

Flour No.	Sample No.	Absorption %	Appearance of loaf	Color of crumb	Texture of crumb	Loaf volume cc.	Baking score	Loaf volume as % of A	Baking score as % of A
	A	64	6	6.5	6.5	242	59	100	100
	B	66	6	7.5	6.5	252	67	104	113
	C	66	6	6.7	6.5	237	60	98	102
1	D	66	6	7.7	6.5	235	61	97	103
	E	64	6	7.5	6.5	245	62	101	105
	F	64	6	8.5	6.5	247	65	102	110
	A	65	8	7	8.5	273	81	100	100
	B	65	8	8	8	277	84	101	104
	C	65	7	8	7.5	267	77	98	95
2	D	65	7	8.5	7.5	240	68	88	84
	E	65	7	8	7.5	275	80	101	99
	F	65	7	9	8	270	82	99	101
	A	67	8.5	8	8.5	310	101	100	100
	B	67	8.5	9	9	307	103	99	102
	C	67	8.5	8.2	8.5	302	98	97	97
3	D	67	8.5	9.5	8	302	99	97	98
	E	67	8.5	8	8	310	100	100	99
	F	67	8.5	9	8	305	100	98	99
	A	65	8	8.5	9	305	99	100	100
	B	65	8	9.2	8.5	300	97	98	98
	C	65	8	9.5	9	302	100	99	101
4	D	65	8	9.5	9	292	96	96	97
	E	65	8	9.2	9.5	292	97	96	98
	F	65	8	10.5	9.5	305	104	100	105
	A	65	8.5	9	9	330	110	100	100
	B	65	8	9.5	9	327	110	99	100
	C	65	8	9.7	8.5	337	113	102	103
5	D	65	8	9.7	9	317	106	96	96
	E	65	8.5	9.5	9	327	110	99	100
	F	65	8.5	10.5	9	347	120	105	109
	A	67	8.5	8.5	8.5	360	122	100	100
	B	67	8	9	9	352	121	98	99
	C	67	8	9.2	9	347	119	96	97
6	D	67	8	10	8.5	347	119	96	97
	E	67	8.5	9.2	8.5	355	121	99	99
	F	67	8.5	10.5	8.5	352	123	98	101
	A	67	9	8.5	8.5	355	120	100	100
	B	67	9	9	7.5	372	125	105	104
	C	67	9	9.5	8	362	124	102	103
7	D	67	9	10	8.5	357	124	101	103
	E	67	9	9	7	355	117	100	97
	F	67	9	10	8	347	119	98	99
	A	65	9	7.5	6.5	387	123	100	100
	B	65	9	8.5	7	395	130	102	106
	C	65	9	9.2	8.5	385	132	99	107
8	D	65	9	9.5	7.5	367	122	95	99
	E	65	9	8.5	7.5	382	126	99	102
	F	65	9	10	8	377	129	97	105

The bromate is used in order that the strong flour may have every possible chance to show its "lifting" ability and we do not believe that the basic formula even approximates this condition with experimentally milled flours.

The baking data obtained on the aged flours by this formula are shown in Table XI.

Looking first at the loaf volume figures, it can be seen that there is a remarkable uniformity of values for the subsamples of each flour. Allowing for manipulative errors a variation of 4%, which amounts to 10 to 16 cc. depending on the size of the loaf, there are only four of the forty bleached samples that vary by more than this amount from their respective check samples. Sample 2D gave a loaf volume 12% lower than its check. This looks as though it had been over bleached. Samples 5F and 7B were both 5% higher than their respective checks and 8D was 5% lower than its check. It is rather doubtful that these differences are very significant.

With regard to the other bread scores, texture shows no systematic variation in the subsamples of any given flour. The color score, however, is lower for the check than for the other subsamples in every case and there is distinct evidence of increase of color score with increasing dosages of each of the bleaching agents. The differences are not very great and thus the baking scores show nearly the same degree of uniformity as the loaf volumes. With the baking score, a greater allowance for manipulative error should be made as errors in scoring enter into the factors making up this value. If we allow a 5% variation only four of the forty bleached samples need be considered different from their respective checks. Baking scores 13%, 6% and 7% higher and 16% lower than their respective checks were given by the subsamples 1B, 8B, 8C and 2D respectively. The increases in the case of flour No. 8 may indicate a significant improvement of strength due to bleaching by Betachlor, although in view of the fact the subsamples 8B and 8C showed no significant increase in loaf volume, it is problematic that a marked increase in "lifting" ability occurred. The 13% increase of 1B and the 16% decrease of 2D both may be considered significant of changes in apparent quality resulting from the treatment with Betachlor.

Summary of Conclusions from the Blend Baking

The most important conclusion that can be drawn from these data is that with flour aged for one month, the treated and untreated samples in general give the same values by the blend formula. With respect to loaf volume, no additional information regarding strength is gained by bleaching. In other words, the sample aged for one month, when baked by the blend formula gives an accurate estimate of the value of the bleached sample aged for the same length of time. The slight loss of information in respect to color is not highly important in a consideration of strength.

These data also seem to justify the conclusion that for estimating relative strength of flours, the unbleached naturally aged samples give nearly maximum values, as the various treatments used in this series gave no higher values in

general. This, together with the observations made by Larmour (7) that the regression of loaf volume on protein of wheat for the blend method is linear for all ranges of protein, indicates that this formula is very valuable in estimating relative strength of hard red spring wheat flours.

Relation of Crude Protein of Wheat and Loaf Volume

It was shown by Larmour (7) that the fraction of the variability of loaf volume independent of protein could be reduced to 18% by using the bromate baking method for determining the loaf volume. It was thought that artificial bleaching might act somewhat similarly to the bromate and "loosen" up the flour protein to the extent that a much better estimate of the actual strength could be obtained. The data presented in Table V indicate that this does occur. In order to get a more accurate idea of the relationship of protein and the results by the various bakings, the correlation coefficients were calculated. These are shown in Table XII.

TABLE XII

COEFFICIENT OF CORRELATION OF LOAF VOLUME AND CRUDE PROTEIN OF WHEAT

Sub-series	r_{xy}				
	1st Basic	1st Bromate	2nd Basic	2nd Bromate	Blend
A	+0.64	+0.68	+0.80	+0.79	+0.96
B	0.48	0.82	0.70	0.83	0.98
C	0.57	0.85	0.70	0.87	0.97
D	0.68	0.86	0.78	0.90	0.94
E	0.75	0.86	0.77	0.86	0.96
F	0.72	0.77	0.63	0.88	0.94

*r	0.62	0.71	0.79	0.83
p	0.1	0.05	0.02	0.01

**Values from Table V.A. (4).*

As there were only eight samples involved in each set of calculations, the significance of the statistics obtained was determined by reference to Table VA (Fisher (4)) in which the ratio $\frac{r}{(1-r^2)^{\frac{1}{2}}} \cdot (n^1-2)^{\frac{1}{2}}$ is used. For small samples this is said to give more reliable results than the value $\frac{r}{(1-r^2)^{\frac{1}{2}}} \cdot \frac{n^{\frac{1}{2}}}{0.6745}$ commonly used. Values of r_{xy} for different levels of significance are appended to Table XII.

With the first baking, loaf volume and protein of wheat are significantly correlated only in the E and F subseries. In all the others, the degree of relationship might have occurred in wholly unrelated population of this size, more than once in twenty times. With values obtained by the blend formula the correlation is high and quite significant in all the subseries. The fraction of the variance of loaf volume not calculable from protein, ranges from 4% to 12%. This indicates that in this series the quantity of protein almost completely determines the loaf volume as estimated by this baking method. The

correlations for the other three bakings are lower than for the blend and higher than for the first basic.

Attention should be directed to the fact that after aging the basic loaf volumes are generally more highly correlated with protein than before, and that with the exception of subseries A and F, aging seems to have little effect on the results by the bromate baking. With the bromate loaf volumes the correlation with protein is higher for the bleached samples than for the unbleached, but this does not hold true for the basic loaf volumes.

Values of the correlation of baking score and protein are given in Table XIII. In general the correlation is highest with the blend data and lowest with the basic data. These data add little to the information gained from the study with loaf volume.

TABLE XIII

Coefficient of correlation of baking score and crude protein of wheat

Sub-series	r_{xy}				
	1st Basic	1st Bromate	2nd Basic	2nd Bromate	Blend
A	+0.79	+0.67	+0.70	+0.71	+0.90
B	0.54	0.77	0.69	0.72	0.94
C	0.60	0.78	0.67	0.77	0.95
D	0.63	0.80	0.75	0.82	0.90
E	0.80	0.82	0.79	0.80	0.92
F	0.83	0.74	0.67	0.80	0.91

*r	0.62	0.71	0.79	0.83
p	0.1	0.05	0.02	0.01

**Values from Table V.A. (4).*

General Summary and Conclusions

A method for gas bleaching whereby a 600-gm. sample of flour can be treated in five minutes has been described.

A series of eight flours of varying protein content was prepared, and subdivided into six subseries. One of these was used as a check, three were bleached with 0.5, 1.0 and 1.5 oz. Betachlor per barrel, respectively and the remaining two were treated with 0.36 and 0.72 oz. Novadel per barrel respectively. The forty-eight samples so obtained were baked the day after bleaching, by the basic and bromate formulas. After storing for one month they were again baked by the basic, bromate and blend formulas.

With these data it was possible to study the effect of the different dosages, the relative effect of bromate and of chlorine, the relative effect of natural and artificial maturing, the relation of protein content to reaction to bleaching agents and the effect of bleaching on the blending value of flours.

The conclusions reached were:—

1. Novadel produces a good bleach but has little if any maturing effect. Betachlor bleaches and matures. The maturing effect on weak flours is negligible but with high protein flours the maturing effect increases with increasing dosage. The reaction seems to be dependent to some extent at least on the quantity of protein present.

2. A comparison of the unbleached flour baked with addition of 1 mg. of $KBrO_3$ and the chlorine-bleached flour baked by the basic method showed that practically the same end result is obtained in both cases. This indicates that the potassium bromate produces with unbleached flour an effect on loaf volume similar to the effect of chlorine. Despite this fact aging improves the chlorine-bleached flours to a certain extent, but not as much as the unbleached or Novadel-bleached flours.

3. Judged from the bread scoring, the color of the check samples aged for one month was practically equal to that of the Novadel-bleached flours immediately after treatment, but slightly inferior to the chlorine-bleached samples (1.5 oz. dosage) immediately after treatment. One of the most interesting observations made in this study was that even with the great improvement in color noticeable one day after treatment, the bleached samples improved further with age to almost the same extent as the unbleached samples. Unfortunately, gasoline color values were determined only on the aged samples so that these observations are subject to the errors incidental to scoring bread color.

4. When these flours were baked by the bromate formula in a blend with very soft flour, no significant difference between the treated and the corresponding untreated check samples could be seen in loaf volume or baking score. The checks gave color scores slightly inferior to the treated samples but this difference was not great enough to materially affect the baking score. From these observations it was concluded that treatment with Betachlor in dosages as high as 1.5 oz. per barrel, and treatment with Novadel in dosages as high as 0.72 oz. per barrel does not significantly affect the blending or "lifting" value of hard red spring wheat flour. These data led also to the conclusion that this blend formula, used with aged untreated flours, would give a quite accurate estimate of the behavior to be expected of the flour when bleached.

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VARIABILITY IN EXPERIMENTAL BAKING
I. THE INFLUENCE OF MECHANICAL MOULDING[1]

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Abstract

In two series of experiments with two different adjustments of the mechanical moulder, 50 hand-moulded and 50 machine-moulded loaves were baked in pairs for each of nine flours of various baking characteristics, using simple and bromate formulas. The data for loaf volume were analyzed statistically. With the first adjustment, machine moulding gave, for the entire series of flours, no significant reduction in variability of loaf volume when an experienced baker conducted the hand moulding. The machine-moulded loaves were significantly lower in loaf volume and exhibited less break and shred, greener crust characteristics, superior grain but inferior texture, and duller crumb color. The mean difference in loaf volume between the hand- and machine-moulded loaves baked from the different flours varied from 28 to 77 cc. or from 4.2 to 9.5% of the corresponding values for hand moulding, the difference increasing with increasing loaf volume. Machine moulding, therefore, reduced the differences between flours and between results for the simple and bromate formulas. Bakers varying in experience secured widely different mean loaf volumes by both hand and machine moulding and there was no evidence of an appreciable reduction in the variability between bakers due to machine moulding. With inexperienced operators, the variability of replicate bakings was slightly lower with machine moulding although greater than for an experienced operator moulding by hand. In general, the less the experience of the baker the lower the loaf volumes obtained. The first adjustment of the moulder did not properly seal doughs made from certain of the flours, and further experiments were undertaken to ascertain the best setting of the machine. Variations in the setting of the sheeting rolls were found to have a more pronounced effect on volume and texture than variations in the adjustment of the compression or former plate. The new setting selected was used in further comparative studies of hand and machine moulding. Replicate tests by an experienced baker using five flours baked by the simple and bromate formulas gave a slight but significant reduction in variability of loaf volume in favor of machine moulding, the coefficient of variability being 2.57% as compared with 2.88% for hand moulding. Replicate bakings of one flour on five consecutive days gave highly significant differences in mean loaf volume for the different days —the range being 18.4 and 27.2 cc. for hand and machine moulding respectively. Arranging the days in order of increasing mean loaf volume the two moulding methods gave a similar arrangement indicating that some factor or factors were operating which affected both the hand and machine results. Significant trends in loaf volume for the baking intervals within any one day were observed. These trends were not similar for the different days with the exception that the last loaves baked were uniformly lower in volume. Machine moulding did not reduce the day differences nor the trends within days, and until the cause of these secular variations is elucidated, they should be taken into account in the planning of baking tests. Experiments conducted by bakers of varying experience gave results which indicated that inexperience in moulding is not an important factor contributing to variability between replicates. The personal factor in both punching and moulding however contributes to the variability between bakers, the data indicating that moulding personality has the greater effect. Using one flour, a range of 61.3 cc. was obtained between the mean loaf volumes for three bakers by hand moulding. When these bakers used doughs which were punched by an experienced operator the range was reduced to 40.1 and 8.7 cc. for hand and machine moulding respectively. From these data it is concluded that machine

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moulding may reduce but not eliminate the large differences in mean loaf volume which different operators secure in baking the same flour. With an experienced experimental baker, the variability of replicates for different flours was found to increase as the mean volume increased. These data are applied to a consideration of reasonable limits for agreement between duplicates and the number of replicate bakings necessary to differentiate between flours.

Introduction

For many years the efforts of investigators in the field of cereal chemistry have been directed mainly towards the determination of those physico-chemical and chemical properties of wheat flour which are responsible for strength, with the ultimate object of finding some simple method whereby baking quality might be accurately predicted. As a result of such studies it has become evident that many factors contribute to the baking quality of a flour and that the quantitative estimation of any one variable does not by itself give a reliable index of flour strength. The interaction of the several contributing and variable factors is such that the baking test is still regarded as the most reliable criterion of flour quality. It is, therefore, not surprising that cereal chemists during the last few years have been devoting more attention to the experimental baking test. The development of this test has recently been adequately reviewed by Brooke and Sherwood (5) and need not be detailed here. The investigations of Werner (16), and Herman and Hart (12) provided the foundation for the development of a baking test based on scientific principles. Blish (2, 3) pointed out the advantages of a fixed baking procedure compared with methods depending on personal judgment and later, as Chairman of the Committee on Standardization of the Experimental Baking Test of the American Association of Cereal Chemists (4), reported the details of the baking procedure proposed.

Before the adoption of the Werner fixed method as the tentative standard procedure of the A.A.C.C., Dunlap (6) and Blish (1) pointed out the importance of the personal factor in the manipulation of doughs, and subsequently Moen (15), reported results which showed that when different operators moulded doughs according to the directions laid down in the standard procedure wide differences in loaf volume and texture were obtained.

In the same year Harrel (11), in presenting the results of the Committee's collaborative work, reported extensive variations in loaf volume, crust color, and external appearance of loaves baked from the same flours by different operators. He stated that "panning personality" was one variable that caused wide variations and suggested that moulding machines might ultimately have to be employed in the standard baking test.

Fifield and Weaver (7) were the first to publish data on the utilization of mechanical moulding in experimental baking. In their experiments the Thomson One-man Moulder Type "I", which is designed to mould the large or commercial size loaf, was modified to render it adaptable to the small experimental doughs. They reported a variation of 5.4% in loaf volume between duplicates by hand moulding, while the maximum variation obtained between duplicates for the machine-moulded doughs was 1.9%. In the instance

of one flour from which 96 loaves were mechanically moulded and baked, the range in loaf volume was 20 cc. with 90% of the loaves falling within a range of ±5 cc. These results indicate that the personal factor in hand moulding is one of the major causes of the variation encountered in replicate bakings.

Until the publication of the recent paper by Treloar and Larmour (16) little definite information was available as to the extent of the variability in loaf volume to be expected in replicate bakes of the same flour. Their results are of considerable interest in connection with the present study. It was found that three experienced operators trained in the same laboratory, using the same equipment, and each baking 50 loaves from the same flour obtained significantly different mean loaf volumes. Moreover, the three bakers showed considerable differences in variability. In the instance of two bakers the total range in loaf volume for the 50 loaves baked was 45 cc., while the third secured a range of 57 cc. On the basis of the normal curve for frequency distribution the theoretical ranges of the central 99% of the distribution were 51.8 and 75.1 cc. for the bakers showing the lowest and the highest variability respectively. This implies that in comparing the average volumes of duplicate bakings of two different flours these bakers would need to secure a difference of 20 and 30 cc. respectively to provide chances of 20 to 1 that the two flours yielded significantly different loaf volumes. Further experiments in which one baker prepared doughs which were subsequently moulded by the three bakers yielded results, in the instance of two bakers, quite similar to those they obtained independently, indicating that "moulding and panning personality" was probably the major factor responsible for the differences between the bakers. Fluctuations in the temperature of the mixed doughs within the range of 28 to 32° C. had no measurable effect on loaf volume and the position of the dough in the proofing cabinet was also found to be without influence.

A study of the factors contributing to variability in experimental baking has interested the Associate Committee on Grain Research of the National Research Council of Canada from the time of its organization. As a result of collaborative work at the Universities of Alberta, Saskatchewan and Manitoba a standard baking procedure based on the Werner fixed method was adopted during the first year. The baking formula, fermentation time, punching schedule and moulding directions were so similar to those subsequently given in the A.A.C.C. tentative method that the latter method was adopted in regard to these particulars. The experimental work in connection with the various projects of the committee is carried out on portions of the same wheat in the three universities and a standard milling and baking procedure is therefore of the utmost importance. The results obtained during the past three years show, in general, that each laboratory secures the same relative baking results on a series of flour samples, but there is considerable discrepancy in the absolute values obtained in the three laboratories on any given flour sample, which greatly complicates the summarizing of the data. In view of these facts, and the opinions expressed in the literature that the personal factor in moulding and panning constituted one of the major causes of variability in experimental

baking, the committee has been interested in the development of a satisfactory machine for the moulding of experimental doughs.

Correspondence with manufacturers of commercial baking machinery brought forth the suggestion from the Thomson Machine Company, Belleville, New Jersey, that the Model G Roll Moulder—a machine of the drum type which they manufactured for the moulding of bread rolls—might prove suitable for the purpose. A test of one of these machines, owned by the Canada Bread Company, Winnipeg, showed that the machine-moulded doughs were slightly too long for the bake pans used in experimental baking. On being advised of this observation, the Thomson Machine Company sent a four-inch straight "former plate" and a test of the machine thus equipped was carried out in January 1930 in the baking laboratory of the Canadian Co-operative Wheat Producers Limited by Mr. Alan MacLeod and the senior author. The machine thus equipped proved quite unsatisfactory, the moulded dough having a poor seal on the long axis and ragged, sticky ends. The moulded loaf was not symmetrical in shape, the ends being much larger than the central portion. The machine-moulded loaves were of smaller volume, lacked break and shred, and possessed an inferior and more variable texture than the hand-moulded loaves. In transmitting these results to the Thomson Machine Company it was suggested that they might be due to the mass of the experimental dough being somewhat more than twice as great as the machine was originally constructed to handle, thus resulting in undue compression. As a result of these preliminary experiments and the interest of the Baking Test Committee of the A.A.C.C. in the development of a laboratory loaf moulder, the Thomson Machine Company made certain constructional changes in the machine and demonstrated their "Laboratory Loaf Moulder" at the Convention of the A.A.C.C. held in Chicago in May 1930. Upon the request of the Associate Committee on Grain Research, a machine was sent on approval for a comparative study of hand and machine moulding.

The experiments reported in this paper were not only planned with this object in view, but also to secure information on the extent of the variability to be expected in the experimental baking of flours yielding different loaf volumes.

Experimental

The baking method used in this study was that adopted by the Associate Committee on Grain Research and is similar, with minor modifications, to the tentative "Basic Standard Procedure" of the A.A.C.C. as outlined by Blish (4). The basic procedure is followed with respect to the proportions of yeast, salt, and sugar, with the difference that 100 gm. of flour on a 13.5% moisture basis (determined by the vacuum oven) is used instead of a 15.0% moisture basis. The absorption is varied in order to produce a dough of the desired consistency, the doughs being mixed in a small Hobart mixer, equipped with two hooks, operated at No. 2 speed for three minutes. The loaves are baked in low-sided pans. The punching schedule, moulding technique, fermentation and proofing time, and baking conditions specified by the A.A.C.C. method are followed.

In previous papers published by the committee the above procedure has been designated as the basic or modified basic method and a similar procedure, with the addition of 1 mg. of potassium bromate to the formula, as the bromate method. Since the actual procedure with regard to mixing, fermentation, proofing, and baking is the same in each case the designation of basic and bromate method does not seem warranted.

It has, therefore, been deemed advisable to differentiate clearly between a mere change in the baking formula only, and an alteration in the baking procedure with regard to fermentation and proofing times. The basic and bromate methods are therefore designated in this paper as the simple formula, standard procedure; and bromate formula, standard procedure, respectively.

In a recent paper, Larmour, Machon, and Brockington (13) have clearly described the baking routine followed in the laboratories collaborating in the work of the Grain Research Committee and have pointed out the importance of reducing temperature differences during fermentation, proofing, and baking. A sectional cabinet similar to that described by these workers, and equipped with rotary shelves was used in this study. A rotary shelf was also placed in the baking oven, and in conducting the experiments reported here close attention was given to the maintenance of uniform temperatures throughout the whole baking procedure. Loaf volume was determined in a measuring device similar to that described by Geddes and Binnington (9). Fifty replicate measurements of a paraffined loaf gave identical readings in 31 cases, the maximum range being 3 cc., indicating that an inappreciable error was to be expected from this source.

While major attention has naturally been given to variability in loaf volume, the effect of machine moulding on other bread characteristics has not been entirely neglected. In each series of experiments, a number of loaves were picked at random, judged and the scores recorded. The method of judging, and the weighting used for the computation of a single figure expressive of bread quality has recently been detailed by Geddes and Winkler (10) and need not be repeated here.

The experiments undertaken are for convenience divided into two series. In each series one adjustment of the machine was used throughout. Since the adjustment selected for the first series did not give satisfactory results with doughs from certain flours, a more detailed study of the setting of the machine was then made and another series of experiments undertaken with the new setting selected.

Series I

I. Experiments to Determine the Optimum Adjustment of the Mechanical Moulder

The Thomson Laboratory Loaf Moulder is of the drum type and relatively simple in principle. It is designed to carry out the moulding of the dough in three operations—sheeting, curling, and compression. The dough first passes through a pair of smooth rolls which remove most of the gas and accomplish the "sheeting". The sheet of dough then passes against a fluted roll which

revolves in the opposite direction to the drum and at a slightly greater surface speed. The dough sheet follows the surface of the drum until it strikes the more rapidly revolving curling roll which causes a partial curling of the front end of the sheet. The curling is then completed between the fluted roll and a flexible curling spring, the dough then passing into the compression chamber. This chamber is formed by the surface of the large rotating drum, the adjustable side walls, and the adjustable compression or "former" plate. In passing through this chamber the dough is turned under pressure by the revolving drum and emerges cylindrical in form. The raw ends of the dough are sealed by the rotary movement against the side walls of the chamber. In the laboratory model there are essentially only two adjustments to be made; namely, the distance between the sheeting rolls and the distance of the former plate from the rotating drum, the latter determining the compression to which the dough is subjected. A hand wheel with indicator and scale is provided for setting the sheeting rolls at any desired adjustment, while the movable former plate is adjusted by means of a hand wheel operating a screw (with 13 threads to the inch) against the former plate.

It became necessary, therefore, to determine the effect of varying these adjustments and to select a setting for further experiments which would give the most satisfactory results. In a series of preliminary trials it was found difficult to measure accurately the distance between the former plate and the drum surface. In order to overcome this difficulty and to ensure that a selected setting could be subsequently obtained the following procedure was adopted: a file scratch was made on one spoke of the adjusting wheel and the reference point for variations in the setting was obtained by rotating the wheel until the length of the exposed bolt between the lock nut and the adjusting handle was $1\frac{5}{16}$ in. with the file notch at the top and in a vertical plane. This setting gave a measurement of 1.28 in. for the depth of the compression chamber at the upper end (where the dough enters) and 1.603 in. at the exit end, the latter measurement being made vertically to the drum. One complete revolution of the adjusting handle altered the depth of the compression chamber at the upper end by $^{1}/_{13}$ in. and by this means it was possible to duplicate any particular setting of the former plate.

For the moulder-setting trials a first patent unbleached flour, commercially milled from hard red spring wheat of the 1929 crop, was used. Two settings of the sheeting rolls with five settings of the former plate were employed, eight loaves being baked by the simple formula, standard procedure, for each setting. In placing the doughs in the moulder, the doughs were carefully removed from the bowl and turned so that the top surface was towards the operator in order that, when rolled, this surface became the outside of the loaf. The amount of dusting flour used corresponded to three strokes of the sifting mechanism. Notes were made on the sealing and shape of the moulded doughs and the grain and texture of the baked loaves examined. The results are summarized in Table I.

When the depth of the compression chamber was 1.43 in., the compression was not sufficient to properly seal the dough along the long axis, resulting in a

deep V-shaped groove on the under side of the baked loaf. Numerous moulding faults were observed with this setting. With the greatest compression employed, the ends of the dough were enlarged, ragged and torn, and the volume of the baked loaf was low. The setting of the sheeting rolls had a pronounced effect on the grain and texture of the baked loaf. In Group *A*, where the breakdown of the dough in the sheeting process was more complete, the cells were round, small, and extremely uniform in size although the cell walls were

TABLE I

Effect of varying the adjustment of the machine

	Group *A* Sheeting rolls set at "2" on dial					Group *C* Sheeting rolls set at "3" on dial				
	Depth of compression chamber at upper end, in inches					Depth of compression chamber at upper end, in inches				
	a 1.43	*b* 1 36	*c* 1.28	*d* 1.21	*e* 1.13	*a* 1.43	*b* 1.36	*c* 1.28	*d* 1.21	*e* 1.13
Mean loaf volume, in cc.	662	670	655	652	640	655	642	641	642	624
Range in loaf volume, in cc.	56	28	50	18	28	30	10	25	33	18
Standard deviation of loaf volume	15.4	10.1	16.3	7.4	8.2	9.2	3.5	6.6	10.8	6.4
Shape of moulded dough	Curved	Slightly curved	Cylindrical	Enlarged ends	Very enlarged ends	Cylindrical, loosely rolled	Cylindrical, loosely rolled	Good	Slightly enlarged ends	Very enlarged ends
Sealing	Poor	Fair	Good	Ragged ends	Very ragged ends	Poor	Good	Good	Fair	Ragged ends
Grain and texture of crumb	9.0c	8.5c	8.5c	7.5c	7.5c	8.0o	8.0o	8.0	8.0	7.5c

Note:—*c* = *coarse*, *o* = *open*.

much thicker than in the case of the *C* setting. With the latter adjustment the cells were more elongated, variable in size and more similar to those yielded by the hand moulding of doughs made from this flour. Setting *C* was selected as giving results most comparable with hand moulding and this adjustment was employed in all subsequent moulder trials of Series I.

II. Machine Versus Hand Moulding by Experienced Operator

In the experimental laboratory, flours varying more widely in handling quality, loaf volume, and texture than those used in commercial bakery practice are encountered and the machine must mould these experimental doughs without any alteration in adjustment to suit the particular characteristics of a given dough. In a study of the value of the machine for experimental

baking it was, therefore, considered essential to compare the results of hand and machine moulding on doughs made from flours of varying characteristics. A suitable range of experimentally milled flours in sufficient quantity to make the required number of replicate bakings was not available and the flours used were, with one exception, commercially milled. The grade, protein and ash content, and the bleaching treatment of the flours selected are recorded in Table II. These flours were milled from hard red spring wheat of the 1929 crop.

TABLE II

Description of flours used in comparison of hand and machine moulding by experienced operator

Flour No.	Description	Bleaching treatment	Chemical analysis, 13.5% moisture basis	
			Protein %	Ash %
1	Commercially milled, 50% patent	Alsop (nitrogen peroxide)	11.9	0.38
2	Commercially milled, bottom 60% less 4% low grade	1.75 gm. Agene (NCl_3) per bbl.+Alsop	13.5	0.51
3	Commercially milled, second clear (bottom 15%)	2.8 gm. Agene (NCl_3) per bbl.+Alsop	15.5	0.90
4	Experimentally milled, (straight grade)+5% corn starch	none	13.5	—

These flours were baked by the standard procedure using both the simple and bromate formulas, except in the case of flour No. 4 where only the simple formula was used. Two hundred grams of flour was mixed at one time, the resulting dough divided into two equal parts by weight and these carried through the baking routine together. One dough was hand moulded and the other machine moulded and both proofed and baked at the same time. The hand- and machine-moulded doughs were thus treated as pairs and were as nearly as possible under similar conditions. Replicate mixings were made at five-minute intervals until the capacity of the fermentation cabinet was reached; after the first pair of doughs went to the oven mixing was resumed. Fifty replicate mixings, using the simple and bromate formulas were made daily on one flour, yielding a total of 200 loaves per day. In order to carry out this routine, one operator attended to the details of mixing, aliquoting and rounding up the doughs, and placing them in the fermentation cabinet. Subsequent operations were attended to entirely by an experienced experimental baker until the loaves came from the oven. Another assistant was required to attend to the measurement of loaf volume, since two loaves had to be measured every five minutes. Ten loaves were selected at random from each lot of fifty and judged the following day. The mean judging scores are given in Table III.

The difference in external characteristics of the hand- and machine-moulded doughs was so pronounced that one could easily separate them when arranged at random. The hand-moulded loaves had in every instance a much bolder appearance with a more pronounced break and shred. The machine-moulded loaves possessed greener crust characteristics, such as sharp corners, and a tendency to a flat top, the crust however was very smooth and uniform and cracked but slightly on cooling. With regard to internal characteristics, there was again a pronounced difference in the crumb of the loaves moulded by the two methods. The machine-moulded loaves were superior in uniformity of cell size—the cells being small and round. The hand-moulded loaves were, however, superior in texture, the crumb possessing greater springiness, and the cells being more elongated and with thinner cell walls. No difference was observed in regard to the variability of these bread characteristics, although moulding faults were more prevalent in the hand-moulded loaves. The crumb color of the machine-moulded loaves was dull and inferior to that of the hand-moulded loaves. This is probably associated with the smaller volume, closer grain and the thicker cell walls of the machine-moulded loaves.

TABLE III

MEAN JUDGING SCORES FOR HAND- AND MACHINE-MOULDED LOAVES BAKED FROM DIFFERENT FLOURS BY AN EXPERIENCED OPERATOR USING THE STANDARD PROCEDURE

Flour No.	Baking formula	External character				Internal character			
		Crust color		Symmetry		Crumb texture		Crumb color	
		Hand	Machine	Hand	Machine	Hand	Machine	Hand	Machine
1	Simple	5.0	4.8p	4.8g	4.5g	7.5c	6.0c	9.0	8.0
1	Bromate	5.0	5.0	4.5–O	5.0	8.0	7.0	9.5	8.5
2	Simple	5.0	4.8p	4.0g	3.5g	5.5c	4.5c	7.5	7.0
2	Bromate	5.0	5.0	5.0	4.5g	7.0o	7.5	8.5	8.0
3	Simple	5.0	4.8	3.0g	2.0g	4.2c	3.2c	2.0dg	1.5dg
3	Bromate	5.0	5.0	4.2g	4.0g	7.0c	5.0c	4.0dg	3.0dg
4	Simple	3.5p	3.2p	3.5g	3.0g	4.0c	3.0c	6.5y	5.8y

NOTE:—*p, pale crust; g, green or underfermented crust character; O, overfermented crust character; c, coarse; o, open; dg, dull grey; y, yellow.*

In the instance of flour No. 3, baked by the simple formula the sealing was not perfect, and the same was noted in many of the loaves baked by the bromate formula from flour No. 2. In the latter case many of the loaves "heaved" away from the bottom of the pan, thus giving a concave surface which caused an error in the measurement of loaf volume. There was also a greater tendency for the machine-moulded loaves to stick to the pans (which were not greased). This was particularly evident with flour No. 3, a low grade flour of poor handling qualities, especially when the doughs were mixed by the simple formula, with the result that several loaves had to be discarded.

TABLE IV

MACHINE VERSUS HAND MOULDING BY EXPERIENCED OPERATOR

SUMMARY OF DATA PERTAINING TO LOAF VOLUMES OBTAINED BY REPLICATE BAKINGS OF FOUR FLOURS BY THE STANDARD PROCEDURE

Flour No.	Baking formula	No. of loaves	Mean loaf volume in cc.						Diff. in L.V. (X−Y) as % L.V. Hand	Range in L. V. in cc.		Std. dev. of loaf volume, in cc.		Sign. of diff. in vari. $Z/\sigma Z$ cc.	Coef. of variability in L. V., %	
			Hand	Machine	Response to bromate: Hand	Response to bromate: Machine	Diff. hand and machine	Sign. of diff. t		Hand	Machine	Hand σx	Machine σy		Hand	Machine
1	Simple	50	676.3	648.1	67.8	62.3	28.2	1.12	4.17	60	60	14.37	15.45	0.508	2.12	2.38
1	Bromate	50	744.1	710.4			33.7		4.53	90	65	18.09	20.72	0.947	2.43	2.92
2	Simple	50	675.0	625.9	209.4	183.6	49.1	4.46	7.27	65	65	13.23	13.46	0.121	1.96	2.15
2	Bromate	50	884.4	809.5			74.9		8.47	120	90	29.68	20.88	2.462	3.36	2.58
3	Simple	46	693.9	633.4	116.6	99.8	60.5	3.03	8.72	75	80	14.77	22.12	2.709	2.13	3.49
3	Bromate	45	810.5	733.2			77.3		9.54	100	60	20.72	16.16	1.649	2.56	2.20
4	Simple	50	540.3	500.9	—	—	39.4	—	7.29	35	30	8.51	7.08	1.288	1.58	1.41
Entire series																
	Simple	196	645.4	601.4			44.0		6.82	210	190	63.27	61.33	0.435	9.80	10.20
	Bromate	145	813.1	751.6	—	—	61.5	—	7.56	240	205	62.84	47.17	3.995	7.73	6.28
	Simple + Bromate	341	716.7	665.3			51.4		7.17	430	370	104.19	92.85	2.124	14.54	13.96

NOTE: x = *loaf volume by hand moulding;* y = *loaf volume by machine moulding.*

Data relating to the loaf volumes obtained for these flours are summarized in Table IV. In every case hand moulding gives a higher loaf volume than machine moulding, the difference being greater when the bromate formula was used. For flours No. 1, 2 and 3 the significance of this difference has been measured and is given by the values of t (Fisher (8)) which should be 2.0 or greater in order to indicate significance. Furthermore, the difference between the hand and machine results becomes greater with flours giving a high loaf volume, thus tending to decrease the absolute differences in volume between the flours.

The variability of loaf volume is of the greatest interest in this study as the purpose of the machine moulder is to reduce variability arising from hand manipulation. The standard deviations are given in Table IV and the significance of the differences between these statistics for hand and machine moulding has been determined by the Z test. The results are given in the column headed $Z/\sigma Z$. If this value is 1.64 or greater the difference may be considered significant. This is the case for flour No. 2, baked by the bromate formula, and flour No. 3, by both formulas, although in the instance of the latter flour baked by the simple formula the variability is greater for machine moulding. Considering the entire series, the absolute variability in loaf volume is significantly lower for machine moulding. This is apparently, in part, due to the lower volume obtained by machine moulding. This in itself tends to lower variability, and when expressed as coefficient of variability, in which the influence of the magnitude of the mean value has been removed, the machine shows but slight advantage. This point will be considered in more detail later.

In order to bring out the salient features of the results of experiments reported here, the analysis of variance as developed by Dr. R. A. Fisher (8) has been adopted as a regular procedure. For each experiment therefore one or more analyses of variance tables have been set up and these, together with tables for the same data giving mean loaf volumes, summarize and furnish the basis for a discussion of the results. In the analysis of variance the total variability of an experiment is expressed as a sum of squares of the deviations of each individual determination from the general mean. This sum of squares is then divided up into component parts and each of these sums of squares may be used to estimate the variability of the experiment by calculating for it a variance (which is the sum of squares divided by the corresponding number of degrees of freedom). The differences between these estimates of the variance bring out the important factors in the experiment and their significance can be measured by means of the Z test (Fisher (8)). For any two estimates of the variance the Z value is one-half the difference between their natural logarithms. The Z value obtained is then compared with the corresponding Z value at the 5% point, which is the value of Z required in order that we may say that the observed difference between the estimates of variance would occur in only 5% of the cases in a large number of trials. This criterion of significance corresponds to odds of about 20 to 1. Reference to Table VIII shows that the total sum of squares, (3,644,066.2), has been divided into nine components.

Similarly the 581 degrees of freedom have been divided into nine components corresponding to the sums of squares. The 581 degrees of freedom arise from the fact that in this experiment 582 loaves were baked and 582 loaf volumes determined (flour No. 4 was omitted from this analysis). The total number of degrees of freedom is always equal to one less than the total number of determinations. The first component is that due to the differences between the flours and since these differences are very large the estimate of variance or mean square is also very large. Continuing we have eight other components in the experiment and the manner in which these are sorted out is best indicated in Table V.

TABLE V

Number of loaves baked for each flour, method of moulding and baking formula

	Simple formula			Bromate formula		
	Flour No. 1	Flour No. 2	Flour No. 3	Flour No. 1	Flour No. 2	Flour No. 3
Hand moulded	1 - - 50	1 - - 50	1 - - 46	1 - - 50	1 - - 50	1 - - 45
Machine moulded	1 - - 50	1 - - 50	1 - - 46	1 - - 50	1 - - 50	1 - - 45

The figures within the cells indicate the number of loaves baked. Flour No. 3 was very sticky and several determinations had to be discarded. In addition to the relations shown by this table the loaves were all baked in pairs, one moulded by hand and one by the machine, so that the only uncontrolled variability was that within each of these pairs and that is the portion given in the table of analysis of variance as random error within pairs. It forms a reliable basis for estimating the random variability in the experiment and may be used therefore to test the significance of the other estimates of variance.

TABLE VI

Number of mean determinations used to obtain the corresponding sum of squares

	Number of mean determinations	Degrees of freedom
Flours	3	2
Hand and machine moulding	2	1
Simple and bromate formula	2	1
Between pairs	291	$285 = (4 \times 49) + 45 + 44$
Within pairs	592	285
Total		574

If the bakings had not been done in pairs the sum of squares for random error would have been greater by the amount corresponding to "between pairs" = 134,100.2, and the error variance would have been 207,367.1/570 = 363.8 instead of 257.1 as given in the table. This gives a rough idea of the increase in precision obtained by planning the experiment so as to control the error.

The degrees of freedom corresponding to the component sums of squares may be obtained by reference to the diagram. First we can set up Table VI, giving the number of mean determinations which are used to obtain the corresponding sum of squares.

For one group such as flour No. 1, simple formula, moulded by hand and by machine there are 50 pairs of loaves and consequently 49 degrees of freedom in this group for "between pairs". Adding the degrees of freedom obtained in this way for all of the groups a grand total of 285 was obtained. Similarly for "within pairs"—each pair consists of two determinations, so that there is one degree of freedom for each pair except the last, and a total of 49 for 50 pairs. Adding again for each group a grand total of 285 was obtained. This accounts so far for only 574 degrees of freedom. The remaining seven represent various interactions between the components, flours No. 1, 2 and 3, hand and machine moulding, and simple and bromate formula. A simple rule for obtaining the interaction degrees of freedom is to multiply the degrees of freedom corresponding to the interacting factors. Thus flours No. 1, 2, 3 (2 D.F.) interacting with hand and machine moulding (1 D.F.) give 2 D.F. for the interaction. For the above case the seven degrees of freedom for interactions are best sorted out as shown in Table VII.

TABLE VII

DEGREES OF FREEDOM FOR INTERACTIONS

	Degrees of freedom
Flours No. 1, 2, 3 × simple and bromate formula	2
Flours No. 1, 2, 3 × hand and machine moulding, simple formula	2
Flours No. 1, 2, 3 × hand and machine moulding, bromate formula	2
Hand and machine moulding × simple and bromate formula	1
	Total = 7

We then have the complete allocation of degrees of freedom as set up in Table VIII.

The mean square or variance column is obtained by dividing the sums of squares by the degrees of freedom on the same line. Then to obtain the Z values each mean square value is compared with that for random error, *i.e.*, each Z value is one-half of the difference between the natural logarithm of the mean square on the same line and the natural logarithm of the mean square for error. The 5% points are obtained from Fisher's tables.

With the above points in mind with regard to analysis of variance tables we can now examine the results given in Tables VIII and XIII-XVI. In Table VIII we note first that the differences between the flours are very significant

and from Table XIV they are placed in the same order for strength when the moulding is done by hand or by machine. Hand moulding results in a much higher average volume than machine moulding. This is a very significant difference as indicated by the relation between a Z value of 3.6942 and a 5% point of 0.6772. The actual mean difference for the entire experiment is 54.0 cc. or about 7.5% of the mean for both methods of moulding. The differences between volumes obtained by the simple and bromate formula are very great. From Table XVI the actual mean difference is 123.3 cc. or 17.1% of the mean for both formulas.

TABLE VIII

MACHINE VERSUS HAND MOULDING BY EXPERIENCED OPERATOR
ANALYSIS OF VARIANCE FOR LOAF VOLUME DATA, HAND AND MACHINE RESULTS COMBINED

Variance due to	Sum of squares	Degrees of freedom	Mean square	Z	5% pt.
Difference between flours No. 1, 2, and 3	293797.6	2	146898.8	3.1741	0.5539
Difference between hand and machine moulding	415735.4	1	415735.4	3.6942	0.6772
Difference between simple and bromate formula	2228014.1	1	2228014.1	4.5336	0.6772
Interaction, flours No. 1, 2, 3, ×simple and bromate formula	447455.8	2	223727.9	3.3844	0.5539
Interaction, flours No. 1, 2, 3, ×hand and machine moulding, simple formula	12967.4	2	6483.7	1.6138	0.5539
Interaction, flours No. 1, 2, 3, ×hand and machine moulding, bromate formula	29445.0	2	14722.5	2.0239	0.5539
Interaction, simple and bromate formula ×hand and machine moulding	9283.8	1	9283.8	1.7933	0.6772
Between pairs	134100.2	285	470.5	0.3023	0.0975
Random error within pairs	73266.9	285	257.1		
Total	3644066.2	581			

The interactions shown in Table VIII are all significant and are of special importance in this study. The interpretation to be put upon a significant interaction is best explained by reference to the simplest case of hand and machine moulding with simple and bromate formula. From Table XIV we can collect the following mean loaf volumes.

TABLE IX

MEAN LOAF VOLUMES FROM TABLE XIV

	Hand moulded, cc.	Machine moulded, cc.	Mean, cc.
Simple formula	681.7	635.8	658.8
Bromate formula	813.0	751.0	782.0
Mean	747.4	693.4	720.4

For hand moulding there is a difference of 131.3 cc. between the volumes for the simple and bromate formula, and for machine moulding this difference is 115.2 cc. Then for the simple formula the difference between the hand and machine results is 45.9 cc., while for the bromate formula this difference is 62.0 cc. The differences seem to be disproportionate and the exact extent of this can be observed by setting up a table of the interaction deviations. For any one determination such as 681.7 cc. in the above table the interaction deviation is given by $681.7+720.4-747.4-658.8=-4.1$. Finally we have a table such as the one below.

TABLE X

Interaction deviations

	Hand moulding	Machine moulding
Simple formula	−4.1	+4.0
Bromate formula	+4.0	−4.0

Applying these corrections to the original values we obtain a set of results showing no interaction. Thus 681.7 is raised to 685.8 so that its interaction deviation is zero.

TABLE XI

Results showing no interaction

	Hand moulding	Machine moulding
Simple formula	685.8	631.8
Bromate formula	809.0	755.0

We now find that for hand moulding the difference between the volumes for the simple and bromate formulas is 123.2 cc. and similarly for machine moulding the difference is 123.2 cc. We have set up theoretical values determined from the means of rows and columns and the extent of the deviations from these theoretical values is the extent of the interaction, the significance of which is tested in the table of the analysis of variance. Evidently the difference between hand and machine results is proportionately greater for the bromate formula than for the simple formula. The same sort of results are indicated for the differences between hand and machine moulding for different flours. These results may be set out as in Table XII.

TABLE XII

Difference between hand and machine moulding and mean values for both methods of moulding

	Simple formula			Bromate formula		
	Flour No. 1	Flour No. 2	Flour No. 3	Flour No. 1	Flour No. 2	Flour No. 3
Difference, hand and machine moulding	28.2	49.1	60.5	33.7	74.9	77.3
Mean for hand and machine moulding	662.2	650.4	663.6	727.2	847.0	771.8

Although flour No. 1, baked with the simple formula is out of line there seems to be some evidence that the greater the volume due either to flour strength or the baking formula, the greater the absolute difference between hand moulding and machine moulding results. Thus the depressing effect on loaf volume of machine moulding, measured in cubic centimetres of loaf volume is greater as the potential strength of the flour increases. In Table XIII the sum of squares for the whole experiment has been divided in a different way to that shown in Table VIII. It amounts simply to dividing the sum of squares into three chief components, one part representing all the loaves moulded by hand, another part representing all the loaves moulded by the machine, and the third part accounting for all the variability due to the difference between the volumes obtained by the two methods. The chief interest in this table lies

TABLE XIII

Machine versus hand moulding by experienced operator

Analysis of variance for loaf volume data, hand and machine results separated

Variance due to	Sum of squares	Degrees of freedom	Mean square	Z	5% pt.
Difference between hand and machine moulding	415735.4	1	415735.4		
Hand moulding					
Difference between flours	244310.1	2	122155.0	2.8858	0.5539
Difference between simple and bromate formula	1262469.6	1	1262469.6	4.0535	0.6772
Interaction, flours No. 1, 2, 3 ×simple and bromate formula	259048.1	2	129524.0	2.9150	0.5539
Random error (x)	108833.2	286	380.54		
Machine moulding					
Difference between flours	89181.3	2	44590.6	2.4280	0.5539
Difference between simple and bromate formula	974828.4	1	974828.4	3.9704	0.6772
Interaction, flours No. 1, 2, 3 ×simple and bromate formula	191126.2	2	95563.1	2.8092	0.5539
Random error (y)	98533.9	284	346.95		
Total	3644066.2	581			

in a comparison of the random errors. We can compare these directly by means of the Z test. The Z value obtained is 0.0462 and since the 5% point is 0.0976 the greater absolute experimental error for the hand results is not significantly different from that for the machine. Furthermore, from the facts which will be pointed out in connection with Part III below, a greater variability and consequently a greater experimental error is expected by the method giving the highest loaf volume. We should, therefore, compare these experimental

TABLE XIV

Machine versus hand moulding by experienced operator
Mean loaf volumes with standard errors based on hand and machine results separated

Method	Flour No. 1	Flour No. 2	Flour No. 3	S.E. of No. 1, 2, or 3	Mean	S.E. of mean
Hand moulding						
Simple	676.3	675.0	693.9	2.76	681.7	1.59
Bromate	744.1	884.4	810.5	2.76	813.0	1.59
Simple+bromate	710.2	779.7	752.2	1.95		
General mean					747.4	1.13
Machine moulding						
Simple	648.1	625.9	633.4	2.63	635.8	1.52
Bromate	710.4	809.5	733.2	2.63	751.0	1.52
Simple+bromate	679.3	717.7	683.3	1.86		
General mean					693.4	1.08

TABLE XV

Machine versus hand moulding by experienced operator
Analysis of variance for loaf volume data, results for simple and bromate formula separated

Variance due to	Sum of squares	Degrees of freedom	Mean square	Z	5% pt.
Difference between simple and bromate formula	2228014.1	1	2228014.1		
Simple formula					
Difference between hand and machine moulding	151082.8	1	151082.8	3.3318	0.6815
Difference between flours No. 1, 2 and 3	10335.3	2	5167.6	1.6441	0.5592
Interaction between flours No. 1, 2, 3 × hand and machine moulding	12967.4	2	6483.7	1.7575	0.5592
Difference between pairs	45053.4	143	315.06	0.2454	0.1380
Random error within pairs (S)	27573.6	143	192.8		
Bromate formula					
Difference between hand and machine moulding	273936.4	1	273936.4	3.3733	0.6815
Difference between flours No. 1, 2 and 3	730918.1	2	365459.0	3.5174	0.5592
Interaction between flours No. 1, 2, 3, × hand and machine moulding	29444.9	2	14722.4	1.9128	0.5592
Difference between pairs	89047.0	142	627.09	0.3335	0.1385
Random error within pairs (B)	45693.1	142	321.78		
Total	3644066.1	581			

errors in relation to the mean loaf volume of the respective experiments. The standard deviation for the hand results is $\sqrt{380.54} = 19.51$ and for the machine results $\sqrt{346.95} = 18.63$. The mean loaf volumes are 747.4 and 693.4 cc. respectively and, expressing the standard deviations in per cent of the corresponding loaf volumes, we have for hand moulding 2.61% and for machine moulding 2.67%.

TABLE XVI

MACHINE VERSUS HAND MOULDING BY EXPERIENCED OPERATOR
MEAN LOAF VOLUMES WITH STANDARD ERRORS BASED ON RESULTS FOR SIMPLE AND BROMATE FORMULA SEPARATED

Method of moulding	Flour No. 1	Flour No. 2	Flour No. 3	S.E. of No. 1, 2 or 3	Mean	S.E. of mean
Simple formula						
Hand	676.3	675.0	693.9	1.96	681.7	1.13
Machine	648.1	625.9	633.4	1.96	635.8	1.13
Hand and machine	662.2	650.4	663.6	1.39		
General mean					658.7	0.80
Bromate formula						
Hand	744.1	884.4	810.5	2.54	813.0	1.46
Machine	710.4	809.5	733.2	2.54	751.0	1.46
Hand and machine	727.2	847.0	771.8	1.79		
General mean					782.0	1.04

Consequently there is no evidence in this experiment to the effect that the use of the machine reduces experimental error with one experienced baker doing the hand moulding.

The experimental error referred to here is quite different from the total variability referred to on page 431. The latter includes for four flours, experimental error or random variability, differences between flours, and differences between results by the simple and bromate formulas. Obviously the total variability is much greater than experimental error and although these totals for four flours were significantly different for the hand and machine results, this does not have any bearing on the differences between the experimental errors for the two methods of moulding. It has been noted that machine moulding reduces differences between flours and between results for the simple and bromate formulas and in view of the comparison of experimental errors as given above the significance of the difference between the total variabilities according to Table IV is obviously almost entirely due to this cause. A similar comparison of total variabilities for flours No. 1, 2 and 3 may be made from the analysis of variance in Table XIII. Total variance for hand moulding is obtained by adding all the sums of squares under this heading and dividing by the sum of the degrees of freedom. We do the same for machine moulding and obtain the following results:

TABLE XVII

Analysis of variance comparing total variability in hand and machine moulding

	Sum squares	Degrees freedom	Variance	Standard deviation	Natural logarithms	Z
Hand moulding	1,874,661.0	291	6442.1	80.26	4.3853	0.1595
Machine moulding	1,353,669.8	289	4684.0	68.44	4.2258	

The 5% point is 0.0970 so that the difference in total variance may be considered significant. Converting the standard deviations to values relative to the corresponding loaf volumes we obtain Table XVIII. This Z value does not exceed the 5% point and is therefore not to be considered significant.

TABLE XVIII

Comparison of relative variability in hand and machine moulding

	Mean loaf volume	Actual standard deviation	Standard deviation relative to loaf volume	Natural logarithms	Z
Hand moulding	747.4	80.26	77.36	4.3485	0.0844
Machine moulding	693.4	68.44	71.10	4.2641	

The results tabulated in Table XIX have been taken from a series of analyses of variance, one for each flour, and from the complete analysis of variance in Table XV. In all these analyses the sums of squares were separated on the basis of results for the simple and bromate formulas. The standard deviations in Table XIX have been obtained by taking the square roots of the mean squares for experimental error and as nearly as possible represent the random errors for each flour, one for the simple formula portion of the experiment and one for the bromate formula portion. These random errors are compared by the Z test. For the three flours combined the bromate formula results give a significantly higher experimental error than those for the simple formula. This is the same result as would be obtained by comparing the random error (S) in Table XV with random error (B).

Table XIX shows also that the significance of the differences between hand and machine loaf volumes are practically the same for either formula and very much the same for different flours.

The correlation coefficients given are intraclass correlation coefficients determined from the relation between the estimates of variance for between pairs and within pairs. They indicate, therefore, the gain in accuracy for the experiments due to the method of baking the loaves in pairs, one hand moulded

TABLE XIX

MACHINE VERSUS HAND MOULDING BY EXPERIENCED OPERATOR

STATISTICAL CONSTANTS PERTAINING TO LOAF VOLUME OBTAINED BY REPLICATE BAKING OF FOUR FLOURS BY THE STANDARD PROCEDURE

Flour No.	Standard deviation		Significance of random error Simple vs. bromate		Significance of difference between hand and machine loaf volumes				Correlation between loaf volumes by hand and machine				$\frac{(Z_1 - Z_2)}{\sigma(Z_1 - Z_2)}$
					Simple		Bromate		Simple		Bromate		
	Simple	Bromate	Z	$Z/\sigma Z$	Z	5% pt.	Z	5% pt.	r_{xy}	$Z_1/\sigma Z_1$	r_{xy}	$Z_2/\sigma Z_2$	
1	13.58	18.09	0.2895+	2.02	2.3412	0.6980	2.2318	0.6980	0.192	1.36	0.151	1.06	
2	10.94	21.74	0.7675+	5.37	3.1911	0.6980	2.8474	0.6980	0.440	3.30	0.296	2.13	
3	17.37	12.14	0.3587−	2.39	2.8141	0.7002	3.4078	0.7009	0.165	1.12	0.583	4.42	
4	6.53	—	—	—	3.4050	0.6980	—	—	0.317	2.30	—	—	
1 & 2 & 3	13.88	17.94	0.2560	3.05	2.8236	0.4907	2.8751	0.4787	0.241	2.93	0.322	3.98	0.75

NOTE:—+ sign indicates bromate formula gives more variable results than the simple formula; *x*, loaf volume by hand moulding; *y*, loaf volume by machine moulding.

TABLE XXII

MACHINE VERSUS HAND MOULDING BY DIFFERENT OPERATORS

SUMMARY OF DATA PERTAINING TO LOAF VOLUMES OBTAINED ON REPLICATE BAKINGS OF FLOUR NO. 2 BY THE SIMPLE FORMULA, STANDARD PROCEDURE

Baker	No. of loaves	Mean loaf volume, cc.			Diff. in L.V. as % L.V. hand	Range in loaf volume, cc.		Std. deviation of L.V., cc.		Significance of diff. in var.	Coefficient of variability in loaf volume, %	
		Hand	Machine	Hand − machine		Hand	Machine	Hand σx	Machine σy	$Z/\sigma Z$	Hand	Machine
A	50	675.0	625.9	49.1	7.27	65	65	13.23	13.46	0.121	1.96	2.15
B	50	621.6	574.4	47.2	7.59	70	70	13.62	12.80	0.435	2.19	2.23
C	50	638.8	615.6	23.2	3.63	80	60	16.25	14.80	0.654	2.54	2.40
D	49	648.7	590.6	58.1	8.96	105	70	23.10	16.91	2.149	3.56	2.86
E	50	614.4	585.1	29.3	4.77	70	50	15.85	11.51	2.240	2.58	1.96
All bakers	249	639.7	598.3	41.4	6.47	115	105	27.21	23.88	2.056	4.25	3.99

and one moulded with the machine. For both formulas, considering the three flours combined, the correlation coefficients are significant and slightly higher for the bromate series. In this case the correlation coefficients can be compared by the difference between their corresponding Z values. The ratio of this difference ($Z_1 - Z_2$) to its standard deviation is only 0.75 so it is not likely that the higher correlation for the series baked by the bromate formula is significant.

III. Machine Versus Hand Moulding by Experienced Operator Duplicate Bakings on a Varied Series of Flours

The observation that the depression of loaf volume as a result of machine moulding was greater as the mean loaf volume increased, coupled with the marked difference noted in the general appearance, crumb texture, and crumb color of the loaves moulded by the two methods led to a comparative study on a larger number of flours to ascertain whether these methods would prove equally valuable in differentiating between the baking quality of the various flours. For this purpose a series of 39 flours were obtained which yielded loaves varying widely in baking characteristics. Twenty-seven of the samples were straight grade flours experimentally milled from wheats of different origin including Western Canadian hard red spring of various grades and varieties, U.S. hard winter, English, French, Hungarian and Australian which were kindly supplied by Mr. Alan Macleod, Chief Chemist of the Canadian Co-operative Wheat Producers' Limited, Winnipeg. Twelve flours commercially milled from hard red spring wheat were included in the series. The procedure of mixing 200 gm. of flour at one time, dividing the dough after mixing, and moulding one dough by hand and the other by machine was employed. The simple formula, standard procedure, was used. After all the samples had been mixed, the series was repeated, thus yielding two loaves moulded by each method. The loaves were scored and a baking score computed using the method described by Geddes and Winkler (10). The mean results are recorded in Table XX.

The same differentiation in the bread characteristics was again observed; namely, the lower loaf volume, lack of boldness, closer grain, coarser texture, and duller crumb color of the machine-moulded loaves, resulting in lower baking scores. The statistical constants calculated for the loaf volumes and baking scores recorded in Table XX are given in Table XXI. While the loaf volumes and baking scores of the individual flours are lower for machine moulding, the high significant positive correlations between the hand and machine values indicate that the two moulding methods give the same relative results for different flours. On the basis of these data, it would appear that, in general, flours may be differentiated in regard to baking quality equally as well by either method of moulding.

Comparing the agreement in loaf volume the average difference between the checks was 19.5 cc. for hand moulding and 16.3 cc. for the machine corresponding to 3.3 and 2.9% of the respective mean loaf volumes. This difference

TABLE XX

MACHINE VERSUS HAND MOULDING BY EXPERIENCED OPERATOR

MEAN RESULTS FOR DUPLICATE BAKINGS BY THE SIMPLE FORMULA, STANDARD PROCEDURE

No.	Origin	Moulding method	Absorption (13.5% moisture basis) %	External character		Internal character		Loaf volume, cc.	Baking score	Difference between duplicate loaf volumes in cc.
				Crust color	Form	Grain and texture	Crumb color			
1	Sask. Ave. 1°	Hand	63	5.0	4.5	7.0o	7.0	635	94	50
	1929 Crop	Machine	63	5.0	3.8	7.0C	6.8	576	82	12
2	Sask. Ave. 2°	Hand	64	4.0p	3.8	6.5c	5.0g	577	77	14
	1929 Crop	Machine	64	4.0p	3.5	5.0C&c	4.8g	544	65	8
3	Sask. Ave. 3°	Hand	64	4.0p	3.8	6.5c	5.0g	605	82	30
	1929 Crop	Machine	64	4.0p	3.5	4.0C	4.5g	570	66	20
4	Canadian 2°	Hand	64	4.0p	4.0	7.0C	5.0g	576	78	12
	1929 Crop	Machine	64	3.0p	3.8	6.0c	5.0g	556	70	28
5	Canadian 2°	Hand	64	4.0p	4.2	7.0o	6.5y	607	88	3
	1929 Crop	Machine	64	3.0p	3.0	6.0c	6.2y	559	72	22
6	U.S. Hard	Hand	61	4.5p	4.5	8.0	6.5y	632	93	3
	Winter, 1929	Machine	61	4.0p	4.0	7.0C	6.0y	594	81	8
7	U.S. Hard	Hand	61	3.0p	3.8	6.0c	6.0y	568	73	40
	Winter, 1929	Machine	61	3.0p	3.0	6.0c	6.0y	539	65	12
8	U.S. Hard	Hand	61	2.0p	2.5s	3.0c	6.0y	491	45	59
	Winter, 929	Machine	61	2.0p	2.5s	3.0c	5.8y	482	42	39
9	Hungarian,	Hand	59	4.0p	3.0	6.2c	5.0g	548	64	7
	1929	Machine	59	4.0p	3.0	4.5C&c	4.5g	539	56	22
10	Swedish	Hand	59	2.0p	3.0	6.2−c	6.0y	507	56	16
	1929	Machine	59	2.0p	3.0	5.0−c	6.0y	493	50	10
11	French	Hand	58	2.5p	3.0	5.5−c	5.5y	508	53	16
	1929	Machine	59	2.5p	3.0	5.5−c	5.0y	509	52	5
12	Australian	Hand	59	3.0p	2.5s	3.0−c	6.0y	450	35	40
	1929	Machine	59	3.0p	2.5s	3.0−c	5.5y	459	36	22
13	Canadian	Hand	69	4.5p	2.8f	3.0−C	4.0g	495	52	10
	No. 6, 1928	Machine	69	4.5p	2.5f	3.0−C	3.5g	475	47	10
14	Canadian	Hand	66	4.5p	3.0f	7.0−o	8.0	609	92	2
	No. 5, 1928	Machine	66	4.0p	3.0f	6.0−c	7.8	568	80	16
15	5th Middlings	Hand	64	5.0	5.0	8.0	8.0	680	110	12
	Flour, 1929	Machine	64	4.5p	4.8	8.0	8.0	611	95	18
16	6th Middlings	Hand	64	5.0	5.0	7.8	8.0	651	103	10
	Flour, 1929	Machine	64	4.5p	5.0	8.0	8.0	625	98	10
17	7th Middlings	Hand	64	5.0	5.0	7.8	8.0	653	103	3
	Flour, 1929	Machine	64	4.0p	4.2	8.0	8.0	602	93	0
18	4th sizer	Hand	64	5.0	3.8f	4.0−C	5.0g&y	597	74	2
	Flour, 1929	Machine	64	4.0d	3.0f	4.0−C	5.0g&y	557	64	6
19	2nd and 3rd	Hand	62	3.5p	3.0f	4.0−C	4.0g&y	562	61	0
	rebolter, 1929	Machine	62	3.5p	3.0f	4.0−C	4.0g&y	524	53	12
20	1st Chip	Hand	65	3.8p	3.8f	3.0−C	3.0g&y	622	72	8
	Flour, 1929	Machine	65	3.8p	3.0f	3.0−C	3.0g&y	578	64	17

TABLE XX—*Continued*

No.	Origin	Moulding method	Absorption (13.5% moisture basis) %	External character: Crust color	External character: Form	Internal character: Grain and texture	Internal character: Crumb color	Loaf volume, cc.	Baking score	Difference between duplicate loaf volumes in cc.
21	3rd Low grade centrifugal Flour	Hand	66	4.5p	3.8f	3.0—C	4.0g&y	600	71	11
		Machine	66	3.8p	3.0f	3.0—C	4.0g&y	566	63	12
22	1st Low grade Flour	Hand	64	4.5p	3.8	6.0—C&o	5.0g&y	593	79	10
		Machine	64	4.0p	3.5	4.0—C	5.0g&y	557	65	2
23	Canadian 2nd Clear (bottom 15%)	Hand	64	4.0d	3.0f	3.5—C	3.0g&y	666	81	12
		Machine	64	4.0d	3.0f	3.0—C	3.0g&y	627	71	14
24	Canadian Bottom 60% (less 4% low grade)	Hand	63	4.5p	4.0	4.0—C	5.0g&y	636	81	12
		Machine	63	4.0p	3.2f	4.0—C	5.0g&y	588	70	8
25	Canadian 1st Patent 1929, 50%	Hand	62	5.0	4.8	8.0	9.0	675	108	34
		Machine	62	4.2p	4.5	7.8	8.0	639	97	8
26	Canadian 1st Patent, 1929	Hand	64	5.0	5.0	7.8	9.0	672	109	14
		Machine	64	4.2p	4.8	8.0	8.0	638	101	12
27	Canadian Ave. 2°, 1929	Hand	68	4.2p	4.0	5.5—C&o	5.0g&y	614	85	40
		Machine	68	4.0p	3.5	5.0—c	4.5g&y	550	70	64
28	Canadian 1°, 1929	Hand	60	3.5p	4.0	6.0—C&o	4.5g&y	559	66	6
		Machine	60	3.5p	3.0f	5.0—c	4.5g&y	515	54	6
29	Manitoba Marquis, 1929	Hand	60	3.5p	4.0	6.5—C&o	5.0g&y	521	61	22
		Machine	60	3.0p	3.5	5.0—c	4.5g&y	502	51	44
30	Manitoba Garnet, 1929	Hand	60	4.0p	4.0	6.8—C&o	2.0 dull	546	64	68
		Machine	60	3.0p	4.0	5.5—C&c	2.0 dull	553	58	10
31	Manitoba Reward, 1929	Hand	60	4.2p	5.0	7.0—o	7.0y	657	95	30
		Machine	60	4.0p	4.5	7.0—o	6.0y	646	85	8
32	Manitoba Marquis, 1929	Hand	59	4.0p	4.0	6.5—C&o	5.0g&y	563	69	6
		Machine	59	3.5p	3.8	6.5—C&c	5.0g&y	557	67	6
33	Manitoba Ceres, 1929	Hand	61	4.5p	4.5	7.0—o	7.0y	579	81	38
		Machine	61	3.8p	4.0	6.5—C&c	7.0y	560	74	32
34	Alberta 1929	Hand	62	3.5p	3.8	5.5—C&o	5.0g&y	556	67	12
		Machine	62	3.5p	3.5	5.5—C&c	5.0g&y	523	60	14
35	Alberta 1929	Hand	62	3.5p	3.8	5.5—C&o	6.0y	539	66	22
		Machine	62	3.5p	3.5	5.0—c	6.0y	529	62	22
36	Alberta 1929	Hand	61	3.5p	4.0	6.0—C&o	5.5y	577	73	34
		Machine	61	3.5p	3.5	6.0—c	5.5y	539	65	38
37	Alberta 1929	Hand	61	4.8p	4.8	7.0—o	8.0	696	106	12
		Machine	61	4.0p	4.2	7.0—o	8.0	647	95	34
38	Alberta 1929	Hand	62	4.5p	4.8	7.0—o	8.0	633	95	14
		Machine	62	4.2p	4.5	7.5	8.0	618	93	4
39	Alberta 1929	Hand	62	3.5p	3.2	4.8—c	6.0y	543	64	26
		Machine	62	3.5p	3.2	4.5—c	6.0y	511	56	2

NOTE:—*Crust color: p, pale; d, dark. Form: f, flat top; s, shell top. Grain and texture: C, coarse; c, close; o, open. Crumb color: g, grey; y, yellow; d, dull.*

in agreement of the duplicates is not significant and hence there is no evidence that machine moulding can be expected, on the average, to produce better agreement between check bakings.

TABLE XXI

MACHINE VERSUS HAND MOULDING BY EXPERIENCED OPERATOR
STATISTICAL CONSTANTS PERTAINING TO LOAF VOLUMES AND BAKING SCORES FOR 39 FLOURS BAKED IN DUPLICATE BY THE SIMPLE FORMULA, STANDARD PROCEDURE

	Hand moulded	Machine moulded
Loaf volume		
Mean loaf volume in cc.	589.7	559.6
Standard deviation of average loaf volume in cc.	58.11	48.10
Mean difference between duplicates in cc.	19.5	16.3
Standard deviation of difference between duplicates in cc.	16.33	13.16
Correlation coefficient (hand × machine)	$+0.958 \pm 0.009$	
Mean difference in loaf volume (hand—machine) in cc.	30.1	
Standard deviation of mean difference in loaf volume in cc.	18.08	
Baking score		
Mean	77.6	68.9
Standard deviation of average baking scores	18.2	16.3
Correlation coefficient (hand × machine)	$+0.978 \pm 0.005$	
Mean difference in baking scores (hand − machine)	8.7	

IV. Machine Versus Hand Moulding by Different Operators

In the experiments so far reported the work was conducted by an experienced operator who had, prior to these studies, been baking regularly fifty loaves per day for several months and the manipulation of the doughs was probably carried out quite uniformly. Our experience has been that a considerable period of training is required before a baker can secure low variability between replicates. The use of the machine would be justified if it were found that bakers with little or no experience could secure a lower variability between replicate bakings of the same flour by machine moulding. A problem of equal importance is the variability between bakers. With the present methods of hand manipulation no two operators, even in the same laboratory, secure the same mean loaf volume on replicate bakings of a given flour. If it were found that by machine moulding the absolute values obtained by different workers showed better agreement, the use of the machine would be amply warranted. Such a result would logically be expected in view of the opinions expressed in the literature that the personal factor in moulding is the chief cause of variation in the results obtained by different bakers.

An experiment was, therefore, planned to determine whether machine moulding would give a lower variability between replicates in the instance of less experienced bakers and to ascertain whether the elimination of the

personal factor in moulding would result in a substantial reduction in the variation between bakers. For this purpose five bakers designated as *A*, *B*, *C*, *D*, *E*, made replicate bakings of flour No. 2 by the simple formula, each moulding fifty loaves by hand and fifty by machine. Baker *A*, who conducted the baking tests previously reported, was the most experienced; *B* had three years' experience but had only baked at irregular intervals for several months; *C* had one month's experience; *D* had rounded up and punched doughs but had never moulded; while *E* had no previous experience whatever.

The means, standard deviations and coefficients of variability are given in Table XXII. We note that in every case hand moulding gave higher loaf volumes than machine moulding. The absolute variability as measured by the standard deviation is also higher for the hand-moulded series for all of the bakers who were inexperienced or out of practice. The experienced baker apparently moulded just as uniformly by hand as with the machine. As indicated by the column headed $Z/\sigma Z$, these differences in absolute variability are significant ($Z/\sigma Z = 1.64$ or more) for bakers *D* and *E*, and for all of the bakers considered together. Variability, however, is related to loaf volume and some differences such as those observed would be expected. But comparing the coefficients of variability the same general trend is evident for the more inexperienced bakers *C*, *D*, and *E*. *A* and *B* show slightly more variability in the machine-moulded series but this is probably insignificant. The average coefficient of variability for all bakers is 2.57 for hand moulding and 2.32 for machine moulding. This average difference of about two-tenths of one per cent represents slightly more than 1 cc. in absolute variability if the mean loaf volume is 600 cc. and therefore even if significant does not represent a very important reduction in variability. Another point worthy of note is that the inexperienced operators using the machine do not obtain as low a variability as the experienced operator moulding by hand.

The analysis of variance Tables XXIV and XXVI were prepared in order to determine the significance of the differences between bakers and whether they obtained the same relative results moulding by hand and with the machine. Table XXIV shows that the differences between the bakers is highly significant and in this respect it must be borne in mind that the bakers worked on different days and, as will be shown later, the same baker working on different days will obtain significantly different results. The differences in this experiment, therefore, are not to be attributed entirely to the individuality of the bakers, and consequently the machine moulder even if producing perfect uniformity in its portion of the experiment would not level out the other and perhaps much greater differences.

An interesting feature of Table XXIV is the significance of the interaction between the bakers and hand and machine moulding. This means that the bakers did not obtain the same relative results in hand and machine moulding. The extent of this may be observed from Table XXV. Taking the loaf volumes expressed in per cent we see that the differences between the hand and machine results are as follows.

TABLE XXIII

Differences between hand and machine results

	Bakers				
	A	*B*	*C*	*D*	*E*
Difference between hand and machine results	7.9	7.6	3.8	9.4	4.7
Mean by both methods of moulding	105.1	96.6	101.3	100.1	96.8

TABLE XXIV

Machine versus hand moulding by different operators

Analysis of variance for loaf volume data, hand and machine results combined

Variance due to	Sum of squares	Degrees of freedom	Mean square	Z	5% pt.
Differences between bakers *A*, *B*, *C*, *D* and *E*	186556.6	4	46639.15	3.0379	0.4446
Differences between hand and machine moulding	212536.0	1	212536.00	3.7962	0.6812
Interaction, bakers *A*, *B*, *C*, *D* and *E* vs. hand and machine moulding	21068.6	4	5267.15	1.9475	0.4446
Differences between pairs	92649.4	244	379.71	0.6325	0.1055
Random error within pairs	26149.4	244	107.17		
Total	538960.0	497			

TABLE XXV

Machine versus hand moulding by different operators

Mean loaf volumes with standard errors

Method of moulding	*A*	*B*	*C*	*D*	*E*	S.E. of *A*, *B*, *C*, *D* or *E*	Mean	S.E. of mean
Hand	675.0	621.6	638.8	648.7	614.4	1.46	639.7	0.66
Machine	625.9	574.4	615.6	590.6	585.1	1.46	598.3	0.66
Hand and machine	650.4	598.0	627.2	619.6	599.8	1.04		
General mean							619.0	0.46
Mean Loaf Volumes and Standard Errors Expressed as a Percentage of the General Mean								
Hand	109.0	100.4	103.2	104.8	99.2	0.24	103.3	0.11
Machine	101.1	92.8	99.4	95.4	94.5	0.24	96.6	0.11
Hand and machine	105.1	96.6	101.3	100.1	96.9	0.17		
General mean							100.0	0.07

The interaction observed is evidently due to some peculiar characteristics of the individual bakers affecting the differences they obtain between results when moulding by hand and by machine. A satisfactory explanation of this fact cannot be given at the present time. This would seem to indicate at first that the more inexperienced bakers *C*, *D* and *E* were more variable in hand moulding, but an examination of the results does not bear this out. Thus *C*, *D*, and *E* obtained by hand moulding the loaf volumes 103.2; 104.8; and 99.2, cc. and by machine moulding they obtained 99.4; 95.4; and 94.5 cc. There is only a slightly greater range in the first case. Then in the analysis set up in Table XXVI we can compare directly the differences between bakers moulding in two ways. The *Z* value is 0.1028 and this does not approach the 5% point which is 0.9272. There is a slight difference as indicated by the mean square values 28612.8 and 23293.6 and if we convert these into standard deviations we have the values 169.15 for hand moulding and 152.62 for machine moulding. In per cent of the mean loaf volumes 639.7 and 598.3 respectively,

TABLE XXVI

MACHINE VERSUS HAND MOULDING BY DIFFERENT OPERATORS

ANALYSIS OF VARIANCE FOR LOAF VOLUME DATA, HAND AND MACHINE RESULTS SEPARATED

Variance due to	Sum of squares	Degrees of freedom	Mean squares	½ log *e*	*Z*	5% pt.
Difference between hand and machine moulding	212536.0	1	212536.0			
Hand moulding						
Differences between *A*, *B*, *C*, *D*, and *E*	114451.0	4	28612.8	5.1308	2.3017	0.4446
Random variability, all bakers	69942.7	244	286.7	2.8291		
Machine moulding						
Differences between bakers *A*, *B*, *C*, *D* and *E*	93174.3	4	23293.6	5.0280	2.3783	0.4446
Random variability, all bakers	48856.0	244	200.2	2.6497		
Total	538960.0	497				

we have 26.4% for hand moulding and 25.5% for machine moulding. This difference is slight and we have, therefore, no evidence of an appreciable reduction in the difference between bakers through the use of a machine moulder.

In the discussion of Table XXII reference was made to the variability within the work of any one baker and the reduction effected by the use of the machine moulder. The analysis of variance in Table XXVI enables us to test the significance of this reduction in variability. For the difference in mean squares

286.7 and 200.2 we obtain the Z value of 0.1794 and the 5% point is 0.1028. The difference though small is significant. The standard deviations are 16.93 and 14.15 for the hand and machine results respectively. In terms of the mean loaf volumes 639.7 and 598.3 these are 2.65% and 2.36%, showing a difference when accurate methods of comparison are used of three-tenths of one per cent.

(To be concluded in next issue.)